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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY DOCKET NO.: 5470-130DV
DATE: February 3, 2000

UTILITY PATENT APPLICATION TRANSMITTAL LETTER AND FEE TRANSMITTAL FORM (37 CFR 1.53(b))

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Transmitted herewith for filing under 37 CFR 1.53(b) is:

☒ a patent application
☐ a Continuation ☒ a Divisional ☐ a Continuation-in-Part (CIP)
of prior application no.: 07/182,646; filed 15 April 1988.
☐ A Small Entity Statement(s) was filed in the prior application; Status still proper and desired.

Inventor(s) or Application Identifier:

Frank S. French, Chapel Hill, North Carolina;
Elizabeth M. Wilson, Chapel Hill, North Carolina
David R. Joseph, Chapel Hill, North Carolina
Dennis B. Lubahn, Columbia, Missouri

Entitled: ***ANDROGEN RECEPTOR PROTEINS, RECOMBINANT DNA MOLECULES CODING FOR SUCH, AND USE OF SUCH COMPOSITIONS***

Enclosed are:

1. ☒ Application Transmittal Letter and Fee Transmittal Form *(A duplicate is enclosed for fee processing)*
2. ☒ 26 pages of Specification (including 6 claims)
3. ☒ 22 sheets of Drawings (35 USC 113)
4. ☐ Oath or Declaration
   a. ☐ newly executed *(original or copy)*
   b. ☐ copy from prior application (37 CFR 1.63(d) *(for continuation/divisional)* **[Note Box 5 Below]**
   c. ☐ DELETION OF INVENTOR(S) *(Signed statement deleting inventor(s) named in the prior application)*
5. ☐ Incorporation By Reference *(useable if box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Microfiche Computer Program *(Appendix)*
7. ☒ Assignment papers *(cover sheet(s) and document(s)* **Filed in parent application**
8. ☐ Small Entity Statement(s)
9. ☒ Information Disclosure Statement, PTO-1449, and 0 of 25 references cited
10. ☒ Preliminary Amendment ***(Please enter all claim amendments prior to calculating the filing fee.)***

11. ☐ English Translation Document
12. ☐ Certified Copy of
13. ☐ Sequence Listing/ Sequence Listing Diskette
    a. ☐ computer readable copy
    b. ☐ paper copy
    c. ☐ statement in support
14. ☐ An Associate Power of Attorney
15. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
16. ☒ Other: Copies of: Decision to Revive Patent Application, Terminal Disclaimer,

The fee has been calculated as shown below:

	Column 1 No. Filed	Column 2 No. Extra	Small Entity Rate Fee	Large Entity Rate Fee
BASIC FEE			$345.00	$690.00
TOTAL CLAIMS	8 - 20 =	0	x 09 = $	x 18 = $
INDEP CLAIMS	2 - 3 =	0	x 39 = $	x 78 = $
☐ MULTIPLE Dependent Claims Presented			+ 130 = $	+ 260 = $
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☒ The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 50-0220.
- ☒ Any additional filing fees required under 37 CFR 1.16.
- ☒ Any patent application processing fees under 37 CFR 1.17.

Respectfully submitted,

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Marjorie J. Pfeiffer
Marjorie J. Pfeiffer
Date of Signature: February 3, 2000

Attorney's Docket No. 5470-130DV PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of Frank S. French et al.
Serial No.: To be assigned
Filed: Concurrently herewith
For: *ANDROGEN RECEPTOR PROTEINS RECOMBINANT DNA MOLECULES AND CODING FOR SUCH USE OF SUCH COMPOSITIONS*

Date : February 3, 2000

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Applicants respectfully request entry of the following amendment in the above-referenced application.

In the Claims:

Please cancel Claims 1-4 for the purposes of rewriting. Please cancel Claims 5 and 6, which were prosecuted in the parent application.

7. An isolated and purified DNA sequence encoding human androgen receptor.

8. The isolated and purified DNA sequence according to claim 7, said receptor having the amino acid sequence set forth in Figure 5.

9. The isolated and purified DNA sequence encoding human androgen receptor and as set forth in Figure 5.

10. The isolated and purified DNA sequence according to claim 9, said DNA sequence having the nucleotide sequence as set forth in Figure 5.

11. A human androgen receptor protein encoded by the DNA according to any one of claim 7 to 10.

12. A prokaryotic or eukaryotic host cell transformed or transfected with a DNA sequence according to any one of claims 7 to 10.

13. A viral or circular DNA plasmid comprising a DNA sequence according to any one of claims 7 to 10.

14. The viral or circular DNA plasmid according to claim 13 further comprising an expression control sequence operatively associated with said DNA sequence.

In the Specification:

Please make the following amendments to the specification.

On page 1 of the application, after the title, add the following:

--**Related Applications**

This application is a divisional of United States Application Serial No. 07/182,646, filed on April 15, 1988, which is hereby incorporated by reference in its entirety.--

REMARKS

The present amendment is submitted to complete the record, to present additional claims for substantive examination, and to provide the basis for an interference with U.S. Patent No. 5,614,620 to Liao et al., issued March 25, 1997.

Pursuant to 37 CFR 1.607(c), it is noted that claims 7-10 and 12-14 presented above correspond to Claims 1 and 6-9 in U.S. Patent No. 5,614,620 to Liao et al. Other claims previously of record, particularly Claim 2 and Claims 7-14 in the parent application, are substantially the same as those submitted in Liao. Claims 2 and 7-14 were of record in the present case prior to or within one year of the issuance of the Liao patent. It is noted that the parent application was filed within three months subsequent to the filing of the Liao patent and, accordingly, falls within the provisions of 37 C.F.R. § 1.608(a).

Applicants respectfully submit that the present application is in condition for substantive examination, which action is respectfully requested.

Respectfully submitted,

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Marjorie J. Pfeiffer
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ANDROGEN RECEPTOR PROTEINS, RECOMBINANT DNA MOLECULES CODING FOR SUCH, AND USE OF SUCH COMPOSITIONS

This invention was made in the course of research supported in part by grants from the National Institutes of Health (NIH HD 16910, HD 04466, and HD 18968).

TECHNICAL FIELD OF THE INVENTION

This invention relates to recombinant DNA molecules and their expression products. More specifically this invention relates to recombinant DNA molecules coding for androgen receptor protein, androgen receptor protein, and use of the DNA molecules and protein in investigatory, diagnostic and therapeutic applications.

BACKGROUND OF THE INVENTION

The naturally occurring androgenic hormones, testosterone and its 5 -reduced metabolite, dihydrotestosterone, are synthesized by the Leydig cells of the testes and circulate throughout the body where they diffuse into cells and bind to the androgen receptor protein ("AR"). Androgens, acting through their receptor, stimulate development of the male genitalia and accessory sex glands in the fetus, virilization and growth in the pubertal male, and maintenance of male virility and reproductive function in the adult. The androgen receptor, together with other steroid hormone receptors constitute a family of trans-acting transcriptional regulatory proteins that control gene transcription through interactions with specific gene sequences.

When prostate cancer is found to be confined to the prostate gland, the treatment of choice is surgical removal. However, 50 to 80% of prostate cancer patients already have metastases at the time of diagnosis. Most of their tumors (70 to 80%) respond to the removal of androgen by castration or by suppression of luteinizing hormone secretion by the pituitary gland using a gonadotropin

releasing hormone analogue alone or in combination with an anti-androgen. The degree and duration of response to this treatment is highly variable (10% live < 6 months, 50% live < 3 years, and 10% live > 10 years.) Initially cancer cells regress without androgen stimulation, but ultimately the growth of androgen independent tumor cells continues (35). At present it is not possible to predict on an individual basis which patient will respond to hormonal therapy and for how long. If poorly responsive patients could be identified early, they could be treated by alternative forms of therapy (e.g. chemotherapy) at an earlier stage when they might be more likely to respond.

Studies on androgen receptors in prostate cancer have suggested that a positive correlation may exist between the presence of androgen receptors in cancer cells and their dependence on androgenic hormone stimulation for growth. (An analogous situation exists in mammary carcinoma where there is a correlation between estrogen receptors and regression of the tumor in response to estrogen withdrawal). However, methodological problems in the measurement of androgen receptors have prevented the routine use of androgen receptor assays in the diagnostic evaluation of prostate cancer. Prior to our preparation of androgen receptor antibodies, all androgen receptor assays were based on the binding of [^{3}H]-labeled androgen. These assays have been unreliable in human prostate cancer tissue because of the extreme lability of the androgen binding site and the presence of unlabeled androgen in the tissue. Endogenous androgen occupies the binding site on the receptor and dissociates very slowly (t 1/2 24-48 hr at 0C). A further problem is that biopsy samples are quite small, making it difficult to obtain sufficient tissue for [^{3}H]-androgen binding assays. Moreover, prostate cancer is heterogenous with respect to cell types. Thus within a single biopsy sample there is likely to be an uneven distribution of cells containing androgen receptors.

Development of the male phenotype and maturation of male reproductive function are dependent on the interaction of androgenic

hormones with the androgen receptor protein and the subsequent function of the receptor as a trans-acting inducer of gene expression. It has become well established over the past twenty-five years that genetic defects of the androgen receptor result in a broad spectrum of developmental and functional abnormalities ranging from genetic males (46,XY) with female phenotype to phenotypically normal males with infertility. Isolation of the structural gene for the androgen receptor makes it possible to define the nature of these genomic defects in molecular terms. Analysis of the functional correlates of the genetic defects may lead to a better understanding of the regulation of androgen receptor gene expression and of the mechanism of androgen action in male sexual development and function.

The androgen insensitivity syndrome, known also as testicular feminization, is characterized by an inability to respond to androgen due to a defect in the androgen receptor, the protein that mediates the action of androgen within the cell. Androgen insensitivity is an inherited X-linked trait that occurs in both complete and incomplete forms. The complete form results in failure of male sex differentiation during embryogenesis and absence of virilization at puberty. The result is a 46,XY genetic male with testes and male internal ducts. The testes produce normal amounts of testosterone and Mullerian inhibiting substance. Consequently development of the uterus is inhibited as in the normal male. Because of the inability to respond to androgen, the external genitalia remain in the female phenotype with normal clitoris and labia. A small vagina develops from the urogenital sinus and ends in a blind pouch. At puberty feminization with breast development and female contours occur in response to testicular estrogen, however, there is no growth of sexual hair even though circulating testosterone concentrations are equal to or greater than levels in the normal male.

Incomplete forms of the androgen insensitivity syndrome include a spectrum of phenotypes resulting from varying degrees of

incomplete androgen responsiveness. At one extreme, individuals have mild enlargement of the clitoris and sparse pubic hair. The opposite extreme is characterized by more complete masculinization with varying degrees of hypospadias deformity but predominantly a male phenotype. It has been reported that some adult men with severe oligospermia or azoospermia who are otherwise normal, have defects in the androgen receptor. These may include as many as 10% of infertile males.

The genetic defect eliciting this range of abnormalities is thought to be a single biochemical event at the level of the gene for the androgen receptor. The androgen receptor is a high affinity androgen binding protein that mediates the effects of testosterone and dihydrotestosterone by functioning as a trans-acting inducer of gene expression. For proper male sexual development to occur, there is a requirement for androgen and its receptor at a critical time during embryogenesis and during puberty. The majority of individuals with the androgen insensitivity syndrome have a history of affected family members, although about a third are thought to represent new mutations of this X-linked disorder. The incidence ranges from 1 in 20,000 to 60,000 male births.

In studies of families with clinical evidence of the androgen insensitivity syndrome, four main categories were recognized that range from the most severe, complete absence of receptor binding activity in a genetic male with female phenotype, to qualitatively normal receptor in affected individuals. Second in severity are affected individuals with qualitatively abnormal androgen binding by receptor present in normal levels. Examples include the failure of sodium molybdate (a reagent often used in studies on steroid receptors) to stabilize the receptor of affected individuals when molybdate is known to stabilize the wild-type receptor. Lability of the receptor under conditions that normally cause transformation has also been reported. A third group expresses a decreased amount of receptor with wild-type in vitro binding characteristics. The final grouping contains those androgen insensitivity patients in

whom no abnormality in receptor is detected. In a recent study of this form of the syndrome, the androgen receptor was as capable of binding oligonucleotides as the wild-type receptor. Indeed, with the techniques available until only recently, it has been difficult in certain cases to document an androgen receptor defect in affected individuals.

Experimental methods used in assessing receptor defects in the past have relied on the ability of receptor to bind androgen with high affinity. The limitation of this methodology is that it is not possible to distinguish between the lack of expression of the receptor and loss of androgen binding activity. An example of how inadequate methodology complicates diagnosis is the absence of detectable receptor binding activity in patients who are partially virilized. It is theoretically possible for a mutation to occur which allows the receptor with defective androgen binding activity to induce gene transcription. Biologically active truncated forms of the glucocorticoid receptor that lack steroid binding activity but retain the DNA binding domain have been demonstrated using genetically engineered mutants.

Purification of the androgen receptor has been difficult to accomplish due to its low concentration and high degree of instability. Reported attempts at purification using either conventional methods of column chromatography or steroid-affinity chromatography have yielded insufficient amounts of receptor protein to allow even the preparation of monoclonal antibodies.

An early report on the partial purification of the androgen receptor was disclosed by Mainwaring et al. in "The use of DNA - cellulose chromatography and isoelectric focusing for the characterization and partial purification of steroid-receptor complexes," Biochem J, 134, 113-127 (1973). They used DNA-cellulose chromatography and isoelectric focusing to isolate the receptor from rat ventral prostate and determined its physiochemical properties. This group was among the first to attempt the use of steroid affinity chromatography in conjunction with conventional

chromatography, using the affinity label 17B-bromoacetoxytestosterone in receptor purification (See Mainwaring et al., "Use of the affinity label 17B-bromoacetoxytestosterone in the purification of androgen receptor proteins," Perspectives in Steroid Receptor Research, (1980)). Partial purification of androgen receptor has also been attempted from other tissue sources, such as ram seminal vesicles (See Foekens et al., Molecular Cellular Endocr, 23, 173-186 (1981) and Foekens et al., "Purification of the androgen receptor of sheep seminal vesicles," Biochem Biophys Res Comm, 104, 1279-1286 (1982)). The partially purified receptor displayed characteristics of a proteolyzed receptor, but a purification of 2,000 fold was reported with a recovery of 33% (See Foekens et al., "Purification of the androgen receptor of sheep seminal vesicles," Biochem Biophys Res Comm, 104, 1279-1286 (1982)). Later attempts at purification continued to combine steroid affinity chromatography with conventional techniques, reportedly achieving significant purification, but recoveries too low for further analysis (See Chang et al., "Purification and characterization of androgen receptor from steer semenal vesicle," Biochemistry 21, 4102-4109 (1982), Chang et al., "Purification and characterization of the androgen receptor from rat ventral prostate," Biochemistry 22, 6170-6175 (1983) and Chang et al., "Affinity labeling of the androgen receptor in rat prostate cytosol with 17B-[(bromoacetyl)oxy]-5-alpha-androstan-3-one," Biochemistry 23, 2527-2533 (1984)). More recent studies examine the effectiveness of a variety of immobilized androgens for their ability to bind the androgen receptor (See De Larminat et al., "Synthesis and evaluation of immobilized androgens for affinity chromatography in the purification of nuclear androgen receptor," The Prostate 5, 123-140 (1984) and Bruchovsky et al, "Chemical demonstration of nuclear androgen receptor following affinity chromatography with immobilized ligands," The Prostate 10, 207-222 (1987)). Despite these efforts, the receptor has not been purified to homogeneity and

the quantities of purified androgen receptor obtained have been insufficient for the production of antisera.

Clinical assays for the androgen receptor now include several methods. The most common is the binding of tritium-labeled hormone and measurement of binding using a charcoal adsorption assay. Either a natural androgen, such as dihydrotestosterone, or synthetic androgen, such as mibolerone or methyltrienolone (R1881), can be used. An advantage of the latter in human tissue is that it is not significantly metabolized and does not bind to the serum androgen binding protein, sex steroid binding globulin. A limitation of radioisotope labeling of receptor is interference caused by endogenous androgen. Although exchange assays for the androgen receptor have been described (See Carroll et al., J Steroid Biochem 21, 353-359 (1984) and Traish et al., J Steroid Biochem 23, 405-413 (1985)), their effectiveness is limited by the slow kinetics of dissociation of the endogenous receptor-bound androgen.

Another method used to assess receptor status is autoradiography. In this method disclosed in Barrack et al., "Current concepts and approaches to the study of prostate cancer," Progress in Clinical and Biological Research, 239, 155-187 (1987) the radioactively labeled androgen is incubated with slide-mounted tissue sections of small tissue biopsy specimens which are then frozen, sectioned and fixed. Nuclear localization of radioactivity is detected by exposure of tissue sections to x-ray film. This technique requires considerable technical expertise, is labor intensive, and requires extended periods of exposure time. It is therefore of limited usefulness in general clinical assays. Another problem is high levels of background signal, i.e. a high noise/signal ratio, making it difficult to distinguish receptor-bound nuclear radioactivity from unbound radioactivity distributed throughout the cells.

WO 87/05049 (Shine) discloses a method for the production of purified steroid receptor proteins, specifically estrogen receptor proteins, through the expression of recombinant DNA encoding for

such proteins in eukaryotic host cells. However, the reference does not disclose the sequence for androgen receptor protein, nor does it disclose a method for obtaining such a sequence.

SUMMARY OF THE INVENTION

The present invention provides a DNA sequence characterized by a structural gene coding for a polypeptide having substantially the same biological activity as androgen receptor protein. A DNA sequence encoding androgen receptor protein or a protein having substantially the same biological activity as androgen receptor activity is also provided. DNA sequences may be obtained from cDNA or genomic DNA, or prepared using DNA synthesis techniques.

The invention further discloses cloning vehicles comprising a DNA sequence comprising a structural gene encoding a polypeptide having substantially the same biological activity as androgen receptor protein. Cloning vehicles comprising a DNA sequence encoding androgen receptor protein or a protein having substantially the same biological activity as androgen receptor protein is also provided. The cloning vehicles further comprise a promoter sequence upstream of and operatively linked to the DNA sequence. In general the cloning vehicles will also contain a selectable marker, and, depending on the host cell used, may contain such elements as regulatory sequences, polyadenylation signals, enhancers and RNA splice sites.

The invention further provides cells transfected or transformed to produce androgen receptor protein or a protein having substantially the same biological activity as androgen receptor protein.

A further aspect of the invention provides a purified androgen receptor protein and purified polypeptides and proteins having substantially the same biological activity as androgen receptor activity, and methods for producing such proteins and polypeptides.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a comparison of DNA-binding domains of the human androgen receptor (hAR) with members of the nuclear receptor family. (A) is a comparison of oligo A nucleotide sequence with sequences of hAR and other nuclear receptors: hPR, human progesterone receptor; hMR, human mineralocorticoid receptor; hGR, human glucocorticoid receptor; hER, human estrogen receptor; hT3R, human thyroid hormone receptor; hRAR, human retinoic acid receptor. Chromosomal locations are shown in parentheses at the left. Nucleotide identity between oligo A and hAR is indicated with an asterisk. The percent homology with oligo A is in parentheses at the right of each sequence. (B) shows the the structure of fibroblast clone ARHFL1 human fibroblast clone [1]). Nucleotide residues are numbered from the 5'-terminus. Restriction endonuclease sites were determined by mapping or were deduced from DNA sequence. The TGA translation termination codon, determined by comparison with hPR, hMR and hGR, follows a long open reading frame containing sequences homologous to those of other steroid receptors. Arrows indicate exon boundaries in genomic clone X05AR. The hatched area is the putative DNA binding domain. (C) shows a comparison of amino acid sequences of the AR DNA-binding domain with sequences of the nuclear receptor family. AR amino acid sequence was deduced from nucleotide sequence of clone ARHFL1 and is numbered beginning with the first conserved cysteine residue (+). Amino acid numbers in parentheses at the left indicate the residue number of the first conserved cysteine from the references indicated above. Percent homology with hAR is indicated in parentheses on the right. The region of the DNA-binding domain from which the oligo A sequence was derived is underlined in hAR. Coding DNA of residues 1 to 31 is contained within genomic clone X05AR. Abbreviations in addition to those described above are cVDR, chicken vitamin D receptor, and vERBA, erb A protein from avian erythroblastosis virus. Abbreviations for amino acid residues are:

A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Figure 2 illustrates the steroid binding properties of expressed AR cDNA. (A) shows the structure of pCMVAR in the expression vector pCMV containing the human cytomegalovirus(CMV) promoter of the immediate early gene, poly(A) addition-transcription terminator region of the human growth hormone gene (hGH poly A),SV40 origin of replication (SV40 Ori), and a polylinker region for insertion of cDNAs. The plasmid pTEBR contains the ampicillin resistance gene (Amp). (B) shows saturation analysis of [^{3}H]dihydrotestosterone binding in extracts of pCMVAR transfection of COS M6 cells. Portions of cytosol (0.1 ml, 0.3 mg/ml protein) were incubated overnight at 4°C with increasing concentrations of ^{3}H-labeled hormone and analyzed by charcoal adsorption. Nonspecific binding increased from 18% to 37% of total bound radioactivity. (C) shows a scratched plot analysis of [^{3}H]dihydrotestosterone binding. Error estimation was based on linear regression analysis (r=0.966). (D) illustrates the competition of unlabeled steroids for binding of 5 nM [^{3}H]dihydrotestosterone in transfected COS M6 cell extracts. Unlabeled steroids were added at 10- and 100-fold excess of labeled hormone. Specific binding was determined as previously described.

Figure 3 is a compiled clone map of the human androgen receptor. The map shows the structure of the human androgen receptor gene and the relative positions of the nucleic acid sequences contained in the cDNA probes [A], [B], [C] and [D], human fibroblast clone [1], human epididymis clones [1] and [5], human genomic clones [1], [2], [3], [4] and [5], and rat epididymis clones [1] and [2].

Figure 4 is a photograph showing chromosome localization of the AR gene on Southern blots of DNA from human cells containing multiple X chromosomes and mouse or hamster cells with X-autosome translocation chromosomes.

Figure 5 shows the complete single strand sequence (5085 bases) of the human androgen receptor and the deduced amino acid sequence. No intron sequence is included.

Figure 6 shows the complete single strand sequence (4260 bases) of the rat androgen receptor and the deduced amino acid sequence.

Figure 7 is a photograph of a frozen section of rat ventral prostate stained with antibodies (AR-52-3-p) to the AR peptide NH_2-Asp-His-Val-Leu-Pro-Ile-Asp-Tyr-Tyr-Phe-Pro-Pro-Gln-Lys-Thr in a dilution of 1 to 3000 using the avidin-biotin peroxidase technique. Androgen receptor is indicated by brown staining of nuclei in epithelial cells.

Figure 8 is a photograph showing restriction fragment length polymorphisms in the human androgen receptor gene.

Figure 9 is a photograph showing a Southern blot analysis in the human androgen receptor gene in complete androgen insensitivity syndrome patients.

DETAILED DESCRIPTION OF THE INVENTION

In the description the following terms are employed:

Nucleotide

A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate,, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. The base characterizes the nucleotide. The four DNA bases are adenine ("A"), guanine ("G"), cytosine ("C") and thymine ("T"). The four RNA bases are A, G, C and uracil ("U"). A and G are purines, abbreviated to R, and C, T, and U are pyrimidines, abbreviated to Y.

DNA Sequence

A linear series of nucleotides connected one to the other by phosphodiester bonds between the 3' and 5' carbons of adjacent pentoses.

Codon

A DNA sequence of three nucleotides (a triplet) which encodes through mRNA an amino acid, a translational start signal or a translational termination signal. For example, the nucleotide triplets TTA, TTG, CTT, CTC, CTA and CTG encode for the amino acid leucine ("Leu"), TAG, TAA and TGA are translational stop signals and ATG is a translational start signal.

Reading Frame

The grouping of codon's during translation of mRNA into amino acid sequences. During translation the proper reading frame must be maintained. For example, the sequence GCTGGTTGTAAG may be translated in three reading frames or phases, each of which affords a different amino acid sequence:

GCT GGT TGT AAG - Ala-Gly-Cys-Lys

G CTG GTT GTA AG - Leu-Val-Val

GC TGG TTG TAA A - Trp-Leu-(STOP)

Polypeptide

A linear series of amino acids connected one to the other by peptide bonds between the a-amino and carboxy groups of adjacent amino acids.

Genome

The entire DNA of a substance. It includes inter alia the structural genes encoding for the polypeptides of the substance, as well as operator, promoter and ribosome binding and interaction sequences including sequences such as the Shine-Dalgarno sequences.

Structural Gene

A DNA sequence which encodes through its template or messenger RNA ("mRNA") a sequence of amino acids characteristic of a specific polypeptide.

Transcription

The process of producing mRNA from a structural gene.

Translation

The process of producing a polypeptide from mRNA.

Expression

The process undergone by a structural gene to produce a polypeptide. It is a combination of transcription and translation.

Plasmid

A non-chromosomal double-stranded DNA sequence comprising an intact "replicon" such that the plasmid is replicated in a host cell. When the plasmid is placed within a unicellular organism, the characteristics of that organism are changed or transformed as a result of the DNA of the plasmid. For example, a plasmid carrying the gene for tetracycline resistance (Tet^R) transforms a cell previously sensitive to tetracycline into one which is resistant to it. A cell transformed by a plasmid is called a "transformant".

Phage or Bacteriophage

Bacterial virus many of which include DNA sequences encapsidated in a protein envelope or coat ("capsid"). In a unicellular organism a phage may be introduced as free DNA by a process called transfection.

Cloning Vehicle

A plasmid, phage DNA or other DNA sequences which are able to replicate in a host cell, characterized by one or a small number of endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without attendant loss of an essential biological function of the DNA, e.g., replication, production of coat proteins or loss of promoter or binding sites, and which contain a marker suitable for use in the identification of transformed cells, e.g., tetracycline resistance or ampicillin resistance. A cloning vehicle is often called a vector.

Cloning

The selection and propagation of a single species.

Recombinant DNA Molecule

A hybrid DNA sequence comprising at least two nucleotide sequences, the first sequence not normally being found together in nature with the second.

Expression Control Sequence

A DNA sequence of nucleotides that controls and regulates expression of structural genes when operatively linked to those genes.

To attain the objects of this invention it was necessary to determine the amino acid sequence and the DNA sequence of the structural gene encoding androgen receptor protein. One conventional approach would involve starting with the purified androgen receptor protein. However, as described above, significant amounts of the protein for such purposes have not been obtained.

An alternative approach to circumvent the overwhelming difficulty of androgen receptor protein purification is direct isolation of the DNA encoding the messenger RNA for androgen receptor protein.

Our strategy for isolating AR DNA was based on evidence that the AR gene is X-linked and that no other steroid receptor gene is located on the X chromosome. Sequence data are available from cDNAs for glucocorticoid , estrogen, progesterone, mineralocorticoid and vitamin D receptors. Comparison of the derived amino acid sequences has revealed a central region of high cysteine content which was found also in the v-erb A oncogene product recently identified as the thyroid hormone receptor. Within this 61-63 amino acid region is an arrangement of 9 cysteine residues that are absolutely conserved among steroid receptors thus far characterized. The overall homology among sequences in this conserved region ranges between 40 and 90%. We assumed that AR would resemble other members of the steroid receptor family in the conserved DNA-binding domain.

A human X chromosomal library was screened with the synthetic oligo nucleotide probe A (Oligo A sequence = $^{5'}$ CTT TTG AAG AAG ACC TTA CAG CCC TCA CAG GT $^{3'}$) of Figure 1 (A) designed as a consensus sequence from the conserved sequence of the DNA-binding domain of other steroid receptors. Screening the library with the oligo A probe resulted in several recombinants whose inserts were cloned into bacteriophage M13 DNA and sequenced. One recombinant

clone (Charon 36 XO5AR) (human genomic clone [1]) contained a sequence similar to, yet distinct from, the DNA-binding domains of other steroid receptors. It had 84% sequence identity with oligo A, while other receptor DNAs were 78% to 91% homologous with the consensus oligonucleotide.

From the nucleotide sequence just 5' of the DNA binding domain, oligonucleotide probe B (Oligo B sequence = ^{5}GGA CCA TGT TTT GCC CAT TGA CTA TTA CTT TCC ACC CC$^{3'}$) was synthesized and used to screen bacteriophage lambda gtll cDNA libraries from human epididymis and cultured human foreskin fibroblasts. Recombinant phage (unamplified) screened with this oligonucleotide by in situ hybridization revealed one positive clone in each library. The epididymal clone (gtll ARHEL1)(human epididymis clone [1]) contained the complete DNA-binding domain and approximately 1.5 kb of upstream sequence, whereas the fibroblast clone (gtll ARHFL1)(human fibroblast clone [1]) shown in Figure 1(B) contained the DNA-binding domain and 1.5 kb of downstream sequence. The DNA-binding domains of the cDNA isolates were identical to that of the genomic exon sequence.

Transient expression in monkey kidney cells (COS M6) demonstrated that the human foreskin fibroblast cDNA fragment encodes the steroid-binding domain of hAR. A DNA fragment (ARHFLIH-X) extending 5' to 3' from the Hind III site within the putative DNA-binding domain through the stop codon (TGA) was cloned into pCMV as shown in Figure 2(A). Expression was facilitated by adding to the 5' end a consensus translation initiation sequence containing the methionine codon (ATG) in reading frame. Transfection of the recombinant construct produced a protein with high-affinity for [^{3}H]dihydrotestosterone, Figure 2(C) saturable at physiological levels of hormone. See Figure 2(B). The binding constant [K_d = 2.7 (+ 1.4) x 10^{-10}M) was nearly identical to that of native AR. The level of expressed protein, 1.3 pmol per milligram of protein, was 20 to 60 times greater than that in male reproductive tissues. Mock transfections without plasmid or

transfections with plasmid DNA lacking th AR insert yielded no specific binding of dihydrotestosterone. Figure 2(D) shows steroid specificity was identical to that of native AR, with highest affinity for dihydrotestosterone and testosterone, intermediate affinity for progesterone and estradiol, and low affinity for cortisol.

Figure 3 is a clone map compiled to show the human androgen receptor gene and the nucleic acid sequences in the cDNA clones, human genomic clones, human fibroblast clones, human epididymis clones, and rat epididymis clones. Human fibroblast clone [1] extended through the stop codon or the C-terminal end of the androgen receptor protein. To isolate and elucidate the sequence of the 5' or N-terminal end of the androgen receptor protein, we used a EcoRl/Sstl fragment (EcoRl site was from the linker) from the 5' end of human epididymis clone [1] as a probe (cDNA probe [A]), to rescreen the human X chromosomal library by standard techniques. By these techniques, human genomic clone [2] was isolated and in turn used as a probe to rescreen a human epididymis library and isolate human epididymis clone [5]. The N-terminal sequence was elucidated along with the 5' flanking sequence of the androgen receptor protein and gene. Human genomic clones [3], [4] and [5] for the sequence 3' of human genomic clone [1] were obtained using cDNA probes B [a Hind III/EcoRl fragment] and C [an EcoRl fragment], by screening and isolating by standard techniques.

Two rat clones, rat epididymis clones [1] and [2], were isolated from a rat epididymis cDNA library using as probes the complete human epididymis clone [1] and a EcoRl/Pstl fragment, cDNA probe [D], respectively. These rat clones contained the entire protein coding sequence for the rat androgen receptor, plus flanking 5' and 3' untranslated sequences which were used to confirm the sequence of the human androgen receptor.

The complete double-stranded sequence encoding the human androgen receptor protein was determined and is set forth in Figure 4. The single-stranded DNA sequence encoding human androgen

receptor protein along with the amino acid sequence which it codes for are set forth in Figure 5. The single stranded DNA sequence and the amino acid sequence for the rat androgen receptor protein is set forth in Figure 6

Recombinant DNA clones human fibroblast clone [1] isolated from human foreskin fibroblast cDNA gt11 expression library, human epididymis clones [1] and [5] isolated from human epididymis cDNA gt11 expression library were deposited in the American Type Culture Collection with accession numbers ATCC # _____,, ATCC # _____ and ATCC # _____ respectively. Human genomic clones [1], [2], [3], [4] and [5] which were isolated from human X chromosome lambda Charon 35 library available as ATCC # 57750 have been deposited with the American Type Culture Collection with accession numbers ATCC # _____ , ATCC # _____, ATCC # _____, ATCC # _____ and ATCC # _____ respectively.

A wide variety of host=cloning vehicle combinations may be usefully employed in cloning the double stranded DNA disclosed herein. For example, useful cloning vehicles may include chroomosomal, non-chromosomal and synthetic DNA sequences such as various known bacterial plasmids and wider host range plasmids such as pCMV and vectors derived from combinations of plasmids and phage DNA such as plasmids which have been modified to employ phage DNA expression control sequences. Useful hosts may include bacterial hosts, yeasts and other fungi, animal or plant hosts, such as Chinese Hamster Ovary cells (CHO, or monkey kidney cells (COS M6), and other hosts. The particular selection of host-cloning vehicle combinations may be made by those of skill in the art after due consideration of factors such as the source of the DNA- i.e. genomic or cDNA.

Cloning vehicles for use in carrying out the present invention will further comprise a promoter operably linked to the DNA sequence encoding the androgen receptor protein. In some instances it is preferred that cloning vehicles further comprise an origin of

replication , as well as sequences which regulate and/or enhance expression levels, depending on the host cell selected.

Techniques for transforming hosts and expressing foreign cloned in them are well known in the art (See, for example, Maniatis et al., infra). Cloning vehicles used for expressing foreign genes in bacterial hosts will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter which functions in the host cell.

Eukaryotic microorganisms, such as the yeast Saccharomyces cerevisiae, may also be used as host cells. Cloning vehicles will generally comprise a selectable marker, such as the nutritional marker TRP, which allows selection in a host strain carrying a trpt mutation. To facilitate purification of an androgen receptor protein produced in a yeast transformant, a yeast gene encoding a secreted protein may be joined to the sequence encoding androgen receptor protein.

Higher eukaryotic cells can also serve as host cells in carrying out the present invention. Cultured mammalian cells are preferred. Cloning vehicles for use in mammalian cells will comprise a promoter capable of directing the transcription of a foreign gene introduced into a mammalian cell. Also contained in the expression vector is a polyadenylation signal, located downstream of the insertion site. The polyadenylation signal can be that of the cloned androgen receptor gene, or may be derived from a heterologous gene.

A selectable marker, such as a gene that confers a selectable phenotype, is generally introduced into the cells along with the gene of interest. Preferred selectable markers include genes that confer resistance to drugs, such as neomycin, hygromycin and methotrexate. Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid.

The copy marker of the integrated gene sequence can be increased through amplification by using certain selectable

markers. Through selection, expression levels may be substantially increased.

Androgen receptor proteins may be purified from the host cells or cell media according to the present invention using techniques well known to those in the art. Such proteins may be utilized to produce monoclonal or polyclonal antibodies according to the techniques described below.

The techniques of this invention offer considerable advances over existing technology for measurement of androgen receptor. Utilizing proteins and peptides containing the disclosed sequences monoclonal or polyclonal antibodies can be produced for use as immunochemical reagents in immunodiagnostic assays. For example, radioimmunoassays and ELISA assays can be developed utilizing these reagents which will allow detection and quantification of androgen receptor in the presence of endogenous androgen since such androgen will not interfere with antibody binding to the receptor.

Immunocytochemistry utilizing our reagents enables determination and quantification of the cellular distribution of the androgen receptor in tumor tissues, which are often heterogenous in composition. This assay offers great potential for diagnostic evaluation of prostate cancer to determine to responsiveness to androgen withdrawal therapy.

In addition, the antibodies produced using the disclosed amino acid sequences can also be used in processes for the purification of androgen receptor protein produced by the above methods. One such purification process is disclosed in Logeat, F., et al., Biochemistry vol. 24 (1985), pp. 1029-1035, which is incorporated by reference herein.

Androgen receptor proteins and polypeptides synthesized from the deduced amino acid sequence can be used as immunogens for the preparation of antibodies to the androgen receptor. Peptides for such use range in length from about 3 to about 958 amino acids in length and are preferrably from about 15 to about 30 amino acids in length. Shorter peptides may have significant sequence homology to

other steroid receptor proteins and larger peptides may contain multiple antigenic determinants; these properties could result in antibodies with cross-reactivities to other steroid receptor proteins.

Peptides can be synthesized from amino acid sequences in the NH_2-terminal region, the DNA-binding domain, and the carboxyl-terminal steroid binding domain. Peptide selection will be based on hydropathic plots, selecting hydrophilic regions that are more likely exposed on the receptor surface. For diagnostic purposes preferred sequences will be selected from the NH_2-terminal region where there is the least homology with other steroid receptor proteins.

Peptides for use as immunogens can be synthesized using techniques available to one of ordinary skill in the art. For example, peptides corresponding to androgen receptor sequences can be synthesized using tBOC chemistry on a Biosearch Model 9500 peptide synthesizer. Peptide purity is assessed by high pressure liquid chromatography. Peptides can be conjugated to keyhole limpet hemocyanin through cysteine residues using the coupling agent m-maleimido-benzoyl-N-hydroxysuccinimide ester. One can also prepare resin-bound peptides utilizing the p-(oxymethyl benzamide) handle to attach the C-terminal amino acid to solid-phase resin support.

Proteins and peptides of this invention can be utilized for the production of poyclonal or monoclonal antibodies. Methods for production of such antibodies are known to those of ordinary skill in the art and may be performed without undue experimentation. One method for the production of monoclonal antibodies is described in Kohler, G., et al., "Continuous Culture of Fused Cells Secreting Antibody of Predefined Specificity," Nature, vol. 256 (1975), p. 495, which is incorporated herein by reference. Polyclonal antibodies, by way of example, can be produced by the method described below.

Peptide conjugates or resin-bound peptides can be injected into rabbits according to the procedure of Vaitukaitis et al., J Clin Endocrinol Metab, 33, 988-991 (1971) using a standard immunization schedule. Antisera titers can be determined in the ELISA assay.

For example, one androgen receptor sequence, NH_2-Asp-His-Val-Leu-Pro-Ile-Asp-Tyr-Tyr-Phe-Pro-Pro-Gln-Lys-Thr in the 5' region upstream from the DNA-binding domain, was used to raise antisera in rabbits. The antisera react selectively at a dilution of 1 to 500 with the androgen receptor both in its untransformed 8-10S form and in its 4-5S transformed form. Receptor sedimentation on sucrose gradients increases from 4 to 8-10S in the presence of antiserum at high ionic strength and from 8-10S to 11-12S at low ionic strength sucrose gradients. In the ELISA reaction against the peptide used as immunogen, reactivity was detectable at 1 to 25,000 dilution. This antiserum at a dilution of 1 to 3000 was found effective in staining nuclear androgen receptor in rat prostate and other male accessory sex glands (see Figure 7).

Our invention provides new molecular probes comprising complementary DNA sequences derived from the deduced sequences encoding the androgen receptor for diagnostic purposes. Such probes may be used to detect the presence of androgen receptor mRNA in tumor cells. Such probes may also be used for detection of androgen receptor gene defects. Androgen receptor complementary DNA sequences can be used as hybridization probes to detect abnormalities in the androgen receptor gene or in its messenger RNA.

Androgen receptor DNA sequences disclosed and complementary RNA sequences can be used to construct probes for use in DNA hybridization assays. An example of one such hybridization assay and methods for constructing probes for such assays are disclosed in U.S.Patent No. 4,683,195 to Mullis et al., U.S. Patent No. 4,683,202 to Mullis, U.S. Patent No. 4,617,261 to Sheldon, III et al., U.S. Patent No. 4,683,194 to Salki et al., and U.S. Patent No. 4,705,886 to Levenson et al., which are hereby incorporated by reference.

By example, one method for detecting gene deletion utilizes Southern blotting and hybridization. DNA can be isolated from cultured skin fibroblasts or from leukocytes obtained from blood. DNA is cut with restriction enzymes, electrophoresed on an agarose gel, blotted onto nitrocellulose, and hybridized with [^{32}P]-labeled androgen receptor DNA (see Maniatis, T. et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, incorporated by reference herein).

In addition, small mutations can be detected utilizing methods known to one of ordinary skill in the art, from cultured skin fibroblasts of the affected individual. A cDNA library can been prepared using standard techniques. The androgen receptor clones can be isolated using a [^{32}PJDNA AR probe. The clones AR cDNA can then be sequenced and compared to normal AR cDNA sequences.

Alternatively genomic DNA can be isolated from blood leukocytes or cultured skin fibroblasts of the affected individual. The DNA is then subjected to restriction enzyme digestion, electrophoresis and is blotted onto nitrocellulose. Synthetic oligonucleotides can be used to bracket specific exons. Exon sequences are amplified using the polymerase chain reaction, cloned into M13 and sequenced. The sequences are compared to normal human AR DNA sequences.

Another method of identifying small mutations or deletions takes advantage of the ability of RNase A to cleave regions of single stranded RNA in RNA:DNA hybrids. Genomic DNA isolated from fibroblasts of affected individuals is hybridized with radioactive RNA probes (Promego Biotec) prepared from wild-type androgen receptor cDNA. Mismatches due to mutations would be cleaved by RNase A and result in altered sized bands relative to wild-type on denaturing polyacrylamide gels.

Restriction fragment length polymorphism (RFLP) linked to the androgen receptor gene locus may be used in prenatal diagnosis and carrier detection of androgen insensitivity. For example, the presence of RFLPs in normal individuals is first established by isolating DNA from lymphocytes of at least six females (total of 12

X chromosomes). DNA can be isolated using the proteinase K procedure and fragmented using a battery of restriction enzymes. Preferred are those enzymes that contain the dinucleotide sequence CG in their recognition sequence. Southern blots are screened with 5-10 kb androgen receptor genomic fragments which if possible lack repetitive DNA. For those regions containing repetitive elements, total human genomic DNA can be added as competitor in the hybridization reaction. Alternatively, one can subclone selected regions to yield a probe free of repetitive elements.

For example, a human restriction fragment length was determined by cDNA probe (B) and Hind III restriction endonuclease using the Southern blot technique (See Figure 8). The two RFLP alleles detected are a fragment at 6.5 kb (allele) and a fragment at 3.5 kb (allele 2). Major constant fragment bands are seen at approximately 2 and 5 kb with minor constant bands at 0.9 and 7.5 kb. Allele 1 is present in approximately 30% of the X chromosomes of the Caucasian population. Allele 2 is present in approximately 20% of the X chromosomes of the Caucasian population. In Figure 9 Lanes A, B and D, DNA from women who are homozygous for allele 1 is shown. In Figure 9 Lane C, DNA from a woman who is heterozygous for both alleles 1 and 2 is shown. Figure 9 Lane E contains DNA from a man that only possesses allele 2. This RFLP, and others determined by the clones we have isolated, will enable one to monitor the androgen receptor gene in various disease conditions described herein.

An example of using the androgen receptor clones to detect mutations is shown in Figure 9 where five different complete androgen insensitive patients' DNA are digested with EcoR1, electrophoresed on a Southern blot, and probed with cDNA probe B. The patient in lane B lacks a 3kb band indicating that part of the androgen receptor gene is deleted. Further analysis of this and other patients DNA is possible with other AR probes and by sequencing by standard methods and comparing the abnormal sequence to the normal sequence described herein.

Other potential uses for oligonucleotide sequences disclosed, for example in construction of therapeutics to block genetic expression, will be obvious to one of ordinary skill in the art.

What is claimed is:

1. A recombinant DNA molecule comprising a DNA sequence encoding the structural gene for androgen receptor protein.

2. The recombinant DNA molecule of Claim 1 wherein the androgen receptor protein is a human androgen receptor protein.

3. A cloning vehicle comprising a genomic DNA molecule which upon expression in a eukaryotic host produces androgen receptor protein.

4. The cloning vehicle of Claim 3 wherein the androgen receptor protein is a human androgen receptor protein.

5. An androgen receptor protein produced by translation of the DNA sequence encoding androgen recptor protein in a host organism transfected or transformed by the cloning vehicle of Claim 3.

6. A human androgen receptor protein produced by translation of the DNA sequence encoding human androgen receptor protein in a host organism transfected or transformed by the cloning vehicle of Claim 4.

ABSTRACT OF THE DISCLOSURE

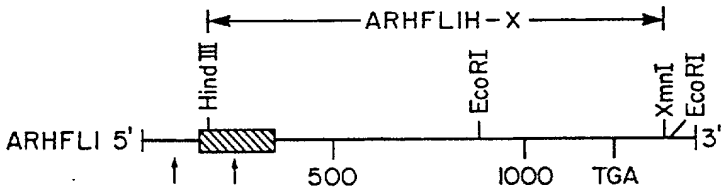
DNA sequences encoding human androgen receptor protein and polypeptides and proteins having substantially the same biological activity as human androgen receptor protein and the amino acid sequences of human androgen receptor protein and polypeptides and proteins having substantially the same biological activity as human androgen receptor protein are disclosed. Methods for the production and use of such compositions are also disclosed.

FIGURE 1

A.

Oligo A Complement	5'-ACC	TGT	GAG	GGC	TGT	AAG	GTC	TTC	TTC	AAA	AG-3'	(100%)
	**	***	*	*	**	***	***	***	***	***	**	
hAR (X)	ACA	TGT	GGA	AGC	TGC	AAG	GTC	TTC	TTC	AAA	AG	(84%)
hPR (11)	ACC	TGT	GGG	AGC	TGT	AAG	GTC	TTC	TTT	AAG	AG	(88%)
hMR (4)	ACC	TGT	GGC	AGC	TGC	AAA	GTT	TTC	TTC	AAA	AG	(81%)
hGR (5)	ACT	TGT	GGA	AGC	TGT	AAA	GTT	TTC	TTC	AAA	AG	(81%)
hER (6)	TCC	TGT	GAG	GGC	TGT	AAG	GCC	TTC	TTC	AAG	AG	(91%)
hT3R (3, 17)	ACG	TGT	GAA	GGC	TGC	AAG	GGT	TTC	TTT	AGA	AG	(78%)
hRAR (17)	GCC	TGT	GAG	GGC	TGC	AAG	GGC	TTC	TTC	CGC	CG	(78%)

B.



C. DNA-Binding Domain

		+	+		10				+	20+						30		
hAR		C	L	I	C	G	D	E	A	S	G	C	H	Y	G	A	L	T C G S C K V F F K R A A E G (100%)
hPR	(aa 567)	C	L	I	C	G	D	E	A	S	G	C	H	Y	G	V	L	T C G S C K V F F K R A M E G (94%)
hMR	(aa 603)	C	L	V	C	G	D	E	A	S	G	C	H	Y	G	V	V	T C G S C K V F F K R A V E G (87%)
hGR	(aa 421)	C	L	V	C	S	D	E	A	S	G	C	H	Y	G	V	L	T C G S C K V F F K R A V E G (87%)
hER	(aa 185)	C	A	V	C	N	D	Y	A	S	G	Y	H	Y	G	V	W	S C E G C K A F F K R S I Q G (55%)
cVDR		C	G	V	C	G	D	R	A	T	G	F	H	F	N	A	M	T C E G C K G F F R R S M K R (48%)
hT3R	(aa 102)	C	V	V	C	G	D	K	A	T	G	Y	H	Y	R	C	I	T C E G C K G F F R R T I Q K (48%)
vERBA	(aa 37)	C	V	V	C	G	D	K	A	T	G	Y	H	Y	R	C	I	T C E G C K S F F R R T I Q K (48%)
hRAR	(aa 58)	C	F	V	C	Q	D	K	S	S	G	Y	H	Y	G	V	S	A C E G C K G F F R R S I Q K (45%)

				+		40		+				50		+		+		60+					
hAR			K	Q	K	Y	L	C	A	S	R	N	D	C	T	I	D	K	F	R	R	K	N C P S C R L R K C Y E A G M (100%)
hPR			Q	H	N	Y	L	C	A	G	R	N	D	C	I	V	D	K	I	R	R	K	N C P A C R L R K C C Q A G M (71%)
hMR			Q	H	N	Y	L	C	A	G	R	N	D	C	I	I	D	K	I	R	R	K	N C P A C R L Q K C L Q A G M (71%)
hGR			Q	H	N	Y	L	C	A	G	R	N	D	C	I	I	D	K	I	R	R	K	N C P A C R Y R K C L Q A G M (71%)
hER			H	N	D	Y	M	C	P	A	T	N	Q	C	T	I	D	K	N	R	R	K	S C Q A C R L R K C Y E V G M (63%)
cVDR			K	A	M	F	T	C	P	F	N	G	D	C	K	I	T	K	D	N	R	R	H C Q A C R L K R C V D I G M (40%)
hT3R		N	L	H	P	S	Y	S	C	K	Y	E	G	K	C	V	I	D	K	V	T	R	N Q C Q E C R F K K C I Y V G M (40%)
vERBA		N	L	H	P	T	T	S	C	T	Y	D	G	C	C	V	I	D	K	I	T	R	N Q C Q L C R F K K C I S V G M (37%)
hRAR			N	M	V	Y	T	C	H	R	D	K	N	C	I	I	N	K	V	T	R	N	R C Q Y C R L Q K C F E V G M (43%)

FIGURE 2

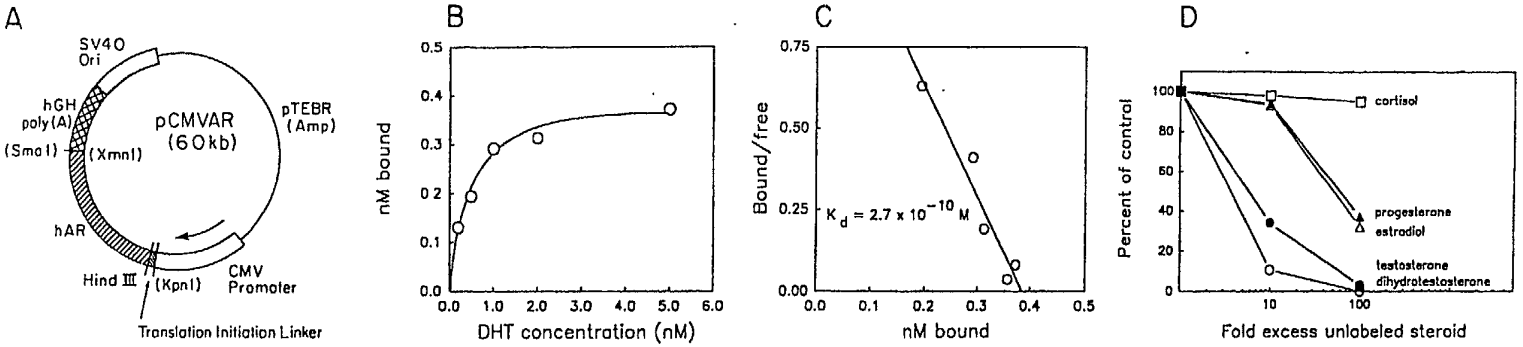


FIGURE 3

Compiled Clone Map of the Human Androgen Receptor

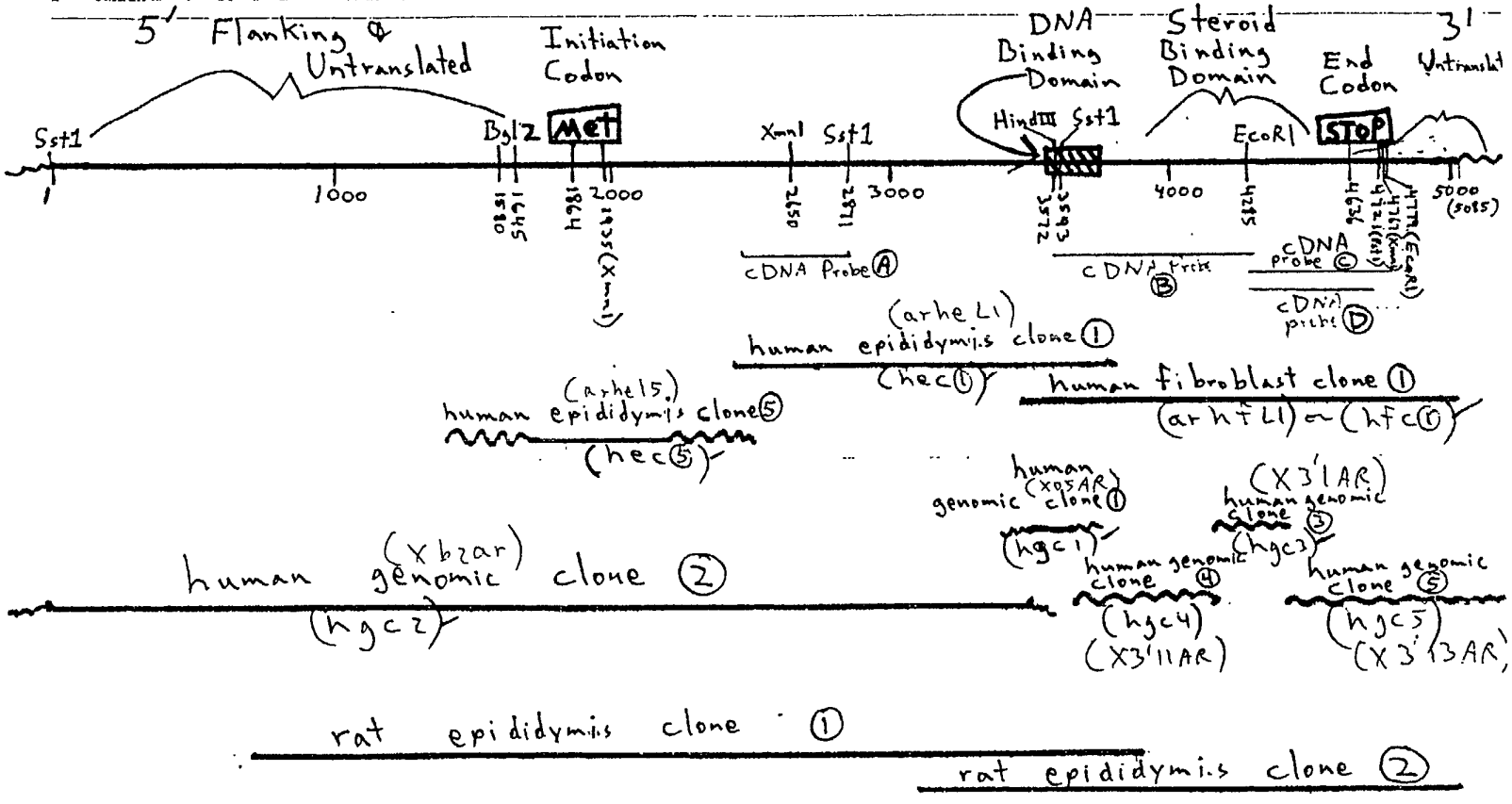


FIGURE 4 (page 1 of 6)

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        10          20          30          40          50          60
GAGCTCTGGA CAAAATTGAG CGCCTATGTG TACATGGCAA GTGTTTTTAG TGTTTGTGTG
CTCGAGACCT GTTTTAACTC GCGGATACAC ATGTACCGTT CACAAAAATC ACAAACACAC

        70          80          90          100         110         120
TTTACCTGCT TGTCTGGGTG ATTTTGCCTT TGAGAGTCTG GATGAGAAAT GCATGGTTAA
AAATGGACGA ACAGACCCAC TAAAACGGAA ACTCTCAGAC CTACTCTTTA CGTACCAATT

        130         140         150         160         170         180
AGGCAATTCC AGACAGGAAG AAAGGCAGAG AAGAGGGTAG AAATGACCTC TGATTCTTGG
TCCGTTAAGG TCTGTCCTTC TTTCCGTCTC TTCTCCCATC TTTACTGGAG ACTAAGAACC

        190         200         210         220         230         240
GGCTGAGGGT TCCTAGAGCA AATGGCACAA TGCCACGAGG CCCGATCTAT CCCTATGACG
CCGACTCCCA AGGATCTCGT TTACCGTGTT ACGGTGCTCC GGGCTAGATA GGGATACTGC

        250         260         270         280         290         300
GAACTCTAAG GTTTCAGCAT CAGCTATCTG CTGGCTTGGT CACTGGCTTG CCTCCTCAGT
CTTGAGATTC CAAAGTCGTA GTCGATAGAC GACCGAACCA GTGACCGAAC GGAGGAGTCA

        310         320         330         340         350         360
TTGTAGGAGA CTCTCCCACT CTCCCATCTG CGCGCTCTTA TCAGTCCTGA AAAGAACCCN
AACATCCTCT GAGAGGGTGA GAGGGTAGAC GCGCGAGAAT AGTCAGGACT TTTCTTGGGN

        370         380         390         400         410         420
TGGCNAGCCA GGAGCNAGGT ATTCNTATCG TCCTTTTCNT CCTCCTNGCC TCACCTNGTT
ACCGNTCGGT CCTCGNTCCA TAAGNATAGC AGGAAAAGNA GGAGGANCGG AGTGGANCAA

        430         440         450         460         470         480
GNTTTTTAGA TTGGNCTTNG NAACCAAATT TGTATGCTGG CCTCCAGGAA ATCTGGAGCC
CNAAAAATCT AACCNGAANC NTTGGTTTAA ACATACGACC GGAGGTCCTT TAGACCTCGG

        490         500         510         520         530         540
TGGCGCCTAA ACCTTGGTTT AGGAAAGCAG GAGCTATTCA GGAAGCAGGG TCCTCCAGGG
ACCGCGGATT TGGAACCAAA TCCTTTCGTC CTCGATAAGT CCTTCGTCCC AGGAGGTCCC

        550         560         570         580         590         600
CTAGAGCTAG CCTCTCCTGC CCTCGCCCAC GTGCGCCAGC ACTTGTTTCT CCAAAGCNAC
GATCTCGATC GGAGAGGACG GGAGCGGGTG CACGCGGTCG TGAACAAAGA GGTTTCGNTG

        610         620         630         640         650         660
TAGGCAGGCG TTAGCGCGCG GTGAGGGGAG GGGAGAAAAG GAAAGGGGAG GGGAGGGAAA
ATCCGTCCGC AATCGCGCGC CACTCCCCTC CCCTCTTTTC CTTTCCCCTC CCCTCCCTTT

        670         680         690         700         710         720
AGGAGGTGGG AAGGCAAGGA GGCCGGCCNG GTGGGGGCGG GACCCGACTC GCANNAACTG
TCCTCCACCC TTCCGTTCCT CCGGCCGGNC CACCCCCGCC CTGGGCTGAG CGTNNTTGAC

        730         740         750         760         770         780
TTGCATTTGC TCTCCACCTC CCAGCGCCCC CTCCGAGATC CCGGGGAGCC AGCTTGCTGG
AACGTAAACG AGAGGTGGAG GGTCGCGGGG GAGGCTCTAG GGCCCCTCGG TCGAACGACC

        790         800         810         820         830         840
GAGAGCGGGA ACGGTCCGGA GCAAGCCCAG AGGCAGAGGA GGCGACAGAG GGAAAAAGGG
CTCTCGCCCT TGCCAGGCCT CGTTCGGGTC TCCGTCTCCT CCGCTGTCTC CCTTTTTCCC

        850         860         870         880         890         900
CCCNAGCTAG CCGCTCCAGT GCTGTACAGN AGCCGAAGGA CGCACCACGC CAGCCCCAGC
GGGNTCGATC GGCGAGGTCA CGACATGTCN TCGGCTTCCT GCGTGGTGCG GTCGGGGTCG
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FIGURE 4 (page 2 of 6)

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       910        920        930        940        950        960
CCGGCTCCAG CGACAGCNAA CGCCTCTTGC ANGCGTTCGA AGCCGCCGCC CGGAGCTGCC
GGCCGAGGTC GCTGTCGNTT GCGGAGAACG TNCGCAAGCT TCGGCGGCGG GCCTCGACGG

       970        980        990       1000       1010       1020
CTTTCCTCTT CGGTGAAGTT TTTAAAAGCT GCTAAAGACT CGGAGGAAGC AAGGAAAGTG
GAAAGGAGAA GCCACTTCAA AAATTTTCGA CGATTTCTGA GCCTCCTTCG TTCCTTTCAC

      1030       1040       1050       1060       1070       1080
CCTGGTAGGA CTGACGGCTG CCTTTGTCCT CCTCCTCTCC ACCCCGCCTC CCCCCACCCT
GGACCATCCT GACTGCCGAC GGAAACAGGA GGAGGAGAGG TGGGGCGGAG GGGGGTGGGA

      1090       1100       1110       1120       1130       1140
GCCTTCCCCC CCTCCCCCGT CTTCTCTCCC GCAGCTGCCT CAGTCGGCTA CTCTCAGCCA
CGGAAGGGGG GGAGGGGGCA GAAGAGAGGG CGTCGACGGA GTCAGCCGAT GAGAGTCGGT

      1150       1160       1170       1180       1190       1200
ACCCCCCTCA CCACCCTTCT CCCCACCCGC CCCCCCGCCC CCGTCGGCCC AGCGNTGNCA
TGGGGGGAGT GGTGGGAAGA GGGGTGGGCG GGGGGGCGGG GGCAGCCGGG TCGCNACNGT

      1210       1220       1230       1240       1250       1260
GNCCGAGTTT GCAGAGAGGT AACTCCCTTT GGCTGCGAGC GGGCGAGNCT AGCTGCACAT
CNGGCTCAAA CGTCTCTCCA TTGAGGGAAA CCGACGCTCG CCCGCTCNGA TCGACGTGTA

      1270       1280       1290       1300       1310       1320
TGCAAAGAAG GCTCTTAGGA GCAGGCGACT GGGGAGCGGC TTCAGCACTG CAGCCACGAC
ACGTTTCTTC CGAGAATCCT CGTCCGCTGA CCCCTCGCCG AAGTCGTGAC GTCGGTGCTG

      1330       1340       1350       1360       1370       1380
CNGCCTGGTT AGGCTGCACG CGGAGAGAAC CCTCTGTTTT CCCCCACTCT CTCTCCACCT
GNCGGACCAA TCCGACGTGC GCCTCTCTTG GGAGACAAAA GGGGGTGAGA GAGAGGTGGA

      1390       1400       1410       1420       1430       1440
CCTCCTGCCT TCCCCACCCC GAGTGCGGAG CCAGAGATCA AAAGATGAAA AGGCAGTCAG
GGAGGACGGA AGGGGTGGGG CTCACGCCTC GGTCTCTAGT TTTCTACTTT TCCGTCAGTC

      1450       1460       1470       1480       1490       1500
GTCTTCAGTA GCCAAAAAAC AAAACAAACA AAAACAAAAA AGCCGAAATA AAAGAAAAAG
CAGAAGTCAT CGGTTTTTTG TTTTGTTTGT TTTTGTTTTT TCGGCTTTAT TTTCTTTTTC

      1510       1520       1530       1540       1550       1560
ATAATAACTC AGTTCTTATT TGCACCTACT TCAGTGGACA CTGAATTTGG AAGGTGGAGG
TATTATTGAG TCAAGAATAA ACGTGGATGA AGTCACCTGT GACTTAAACC TTCCACCTCC

      1570       1580       1590       1600       1610       1620
ATTTTGTTTT TTTCTTTTAA GATCTGGGCA TCTTTTGAAT CTACCCTTCA AGTATTAAGA
TAAAACAAAA AAAGAAAATT CTAGACCCGT AGAAAACTTA GATGGGAAGT TCATAATTCT

      1630       1640       1650       1660       1670       1680
GACAGACTGT GAGCCTAGCA GGGCAGATCT TGTCCACCGT GTGTCTTCTT CTGCACGAGA
CTGTCTGACA CTCGGATCGT CCCGTCTAGA ACAGGTGGCA CACAGAAGAA GACGTGCTCT

      1690       1700       1710       1720       1730       1740
CTTTGAGGCT GTCAGAGCGC TTTTTGCGTG GTTGCTCCCG CAAGTTTCCT TCTCTGGAGC
GAAACTCCGA CAGTCTCGCG AAAAACGCAC CAACGAGGGC GTTCAAAGGA AGAGACCTCG

      1750       1760       1770       1780       1790       1800
TTCCCGCAGG TGGGCAGCTA GCTGCAGCGA CTACCGCATC ATCACAGCCT GTTGAACTCT
AAGGGCGTCC ACCCGTCGAT CGACGTCGCT GATGGCGTAG TAGTGTCGGA CAACTTGAGA
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FIGURE 4 (page 3 of 6)

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      1810        1820        1830        1840        1850        1860
TCTGAGCAAG AGAAGGGGAG GCGGGGTAAG GGAAGTAGGT GGAAGATTCA GCCAAGCTCA
AGACTCGTTC TCTTCCCCTC CGCCCCATTC CCTTCATCCA CCTTCTAAGT CGGTTCGAGT

      1870        1880        1890        1900        1910        1920
AGGATGGAAG TGCAGTTAGG GCTGGGAAGG GTCTACCCTC GGCCGCCGTC CAAGACCTAC
TCCTACCTTC ACGTCAATCC CGACCCTTCC CAGATGGGAG CCGGCGGCAG GTTCTGGATG

      1930        1940        1950        1960        1970        1980
CGAGGAGCTT TCCAGAATCT GTTCCAGAGC GTGCGCGAAG TGATCCAGAA CCCGGGCCCC
GCTCCTCGAA AGGTCTTAGA CAAGGTCTCG CACGCGCTTC ACTAGGTCTT GGGCCCGGGG

      1990        2000        2010        2020        2030        2040
AGGCACCCAG AGGCCGCGAG CGCAGCACCT CCCGGCGCCA GTTTGCTGCT GCTGCAGCAG
TCCGTGGGTC TCCGGCGCTC GCGTCGTGGA GGGCCGCGGT CAAACGACGA CGACGTCGTC

      2050        2060        2070        2080        2090        2100
CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG
GTCGTCGTCG TCGTCGTCGT CGTCGTCGTC GTCGTCGTCG TCGTCGTCGT CGTCGTCGTC

      2110        2120        2130        2140        2150        2160
CAGCAGCAAG AGACTAGCCC CAGGCAGCAG CAGCAGCAGC AGGGTGAGGA TGGTTCTCCC
GTCGTCGTTC TCTGATCGGG GTCCGTCGTC GTCGTCGTCG TCCCACTCCT ACCAAGAGGG

      2170        2180        2190        2200        2210        2220
CAAGCCCATC GTAGAGGCCC CACAGGCTAC CTGGTCCTGG ATGAGGAACA GCAACCTTCA
GTTCGGGTAG CATCTCCGGG GTGTCCGATG GACCAGGACC TACTCCTTGT CGTTGGAAGT

      2230        2240        2250        2260        2270        2280
CAGCCGCAGT CGGCCCTGGA GTGCCACCCC GAGAGAGGTT GCGTCCCAGA GCCTGGAGCC
GTCGGCGTCA GCCGGGACCT CACGGTGGGG CTCTCTCCAA CGCAGGGTCT CGGACCTCGG

      2290        2300        2310        2320        2330        2340
GCCGTGGCCG CCAGCAAGGG GCTGCCGCAG CAGCTGCCAG CACCTCCGGA CGAGGATGAC
CGGCACCGGC GGTCGTTCCC CGACGGCGTC GTCGACGGTC GTGGAGGCCT GCTCCTACTG

      2350        2360        2370        2380        2390        2400
TCAGCTGCCC CATCCACGTT GTCCCTGCTG GGCCCCACTT TCCCCGGCTT AAGCAGCTGC
AGTCGACGGG GTAGGTGCAA CAGGGACGAC CCGGGGTGAA AGGGGCCGAA TTCGTCGACG

      2410        2420        2430        2440        2450        2460
TCCGCTGACC TTAAAGACAT CCTGAGCGAG GCCAGCACCA TGCAACTCCT TCAGCAACAG
AGGCGACTGG AATTTCTGTA GGACTCGCTC CGGTCGTGGT ACGTTGAGGA AGTCGTTGTC

      2470        2480        2490        2500        2510        2520
CAGCAGGAAG CAGTATCCGA AGGCAGCAGC AGCGGGAGAG CGAGGGAGGC CTCGGGGGCT
GTCGTCCTTC GTCATAGGCT TCCGTCGTCG TCGCCCTCTC GCTCCCTCCG GAGCCCCCGA

      2530        2540        2550        2560        2570        2580
CCCACTTCCT CCAAGGACAA TTACTTAGGG GGCACTTCGA CCATTTCTGA CAACGCCAAG
GGGTGAAGGA GGTTCCTGTT AATGAATCCC CCGTGAAGCT GGTAAAGACT GTTGCGGTTC

      2590        2600        2610        2620        2630        2640
GAGTTGTGTA AGGCAGTGTC GGTGTCCATG GGCCTGGGTG TGGAGGCGTT GGAGCATCTG
CTCAACACAT TCCGTCACAG CCACAGGTAC CCGGACCCAC ACCTCCGCAA CCTCGTAGAC

      2650        2660        2670        2680        2690        2700
AGTCCAGGGG AACAGCTTCG GGGGGATTGC ATGTACGCCC CACTTTTGGG AGTTCCACCC
TCAGGTCCCC TTGTCGAAGC CCCCCTAACG TACATGCGGG GTGAAAACCC TCAAGGTGGG
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FIGURE 4 (page 4 of 6)

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      2710        2720        2730        2740        2750        2760
GCTGTGGCTC CCACTCCTTG TGCCCCATTG GCCGAATGCA AAGGTTCTCT GCTAGACGAC
CGACACCGAG GGTGAGGAAC ACGGGGTAAC CGGCTTACGT TTCCAAGAGA CGATCTGCTG

      2770        2780        2790        2800        2810        2820
AGCCCAGGCA AGAGCACTGA AGATACTGCT GAGTATTCCC CTTTCAAGGG AGGTTACACC
TCGCGTCCGT TCTCGTGACT TCTATGACGA CTCATAAGGG GAAAGTTCCC TCCAATGTGG

      2830        2840        2850        2860        2870        2880
AAAGGGCTAG AAGGCGAGAG CCTAGGCTGC TCTGGCAGCG CTGCAGCAGG GAGCTCCGGG
TTTCCCGATC TTCCGCTCTC GGATCCGACG AGACCGTCGC GACGTCGTCC CTCGAGGCCC

      2890        2900        2910        2920        2930        2940
ACACTTGAAC TGCCGTCTAC CCTGTCTCTC TACAAGTCCG GAGCACTGGA CGAGGCAGCT
TGTGAACTTG ACGGCAGATG GGACAGAGAG ATGTTCAGGC CTCGTGACCT GCTCCGTCGA

      2950        2960        2970        2980        2990        3000
GCGTACCAGA GTCGCGACTA CTACAACTTT CCACTGGCTC TGGCCGGACC GCCGCCCCCT
CGCATGGTCT CAGCGCTGAT GATGTTGAAA GGTGACCGAG ACCGGCCTGG CGGCGGGGGA

      3010        3020        3030        3040        3050        3060
CCGCCGCCTC CCCATCCCCA CGCTCGCATC AAGCTGGAGA ACCCGCTGGA CTACGGCAGC
GGCGGCGGAG GGGTAGGGGT GCGAGCGTAG TTCGACCTCT TGGGCGACCT GATGCCGTCG

      3070        3080        3090        3100        3110        3120
GCCTGGGCGG CTGCGCGGCC GCACTGCCGC TATGGGGACC TGGCGAGCCT GCATGGCGCG
CGGACCCGCC GACGCCGCCG CGTGACGGCG ATACCCCTGG ACCGCTCGGA CGTACCGCGC

      3130        3140        3150        3160        3170        3180
GGTGCAGCGG GACCCGGTTC TGGGTCACCC TCAGCCGCCG CTTCCTCATC CTGGCACACT
CCACGTCGCC CTGGGCCAAG ACCCAGTGGG AGTCGGCGGC GAAGGAGTAG GACCGTGTGA

      3190        3200        3210        3220        3230        3240
CTCTTCACAG CCGAAGAAGG CCAGTTGTAT GGACCGTGTG GTGGTGGTGG GGGTGGTGGC
GAGAAGTGTC GGCTTCTTCC GGTCAACATA CCTGGCACAC CACCACCACC CCCACCACCG

      3250        3260        3270        3280        3290        3300
GGCGGCGGCG GCGGCGGCGG CGGCGGCGGC GGCGGCGGCG GCGGCGGCGG CGAGGCGGGA
CCGCCGCCGC CGCCGCCGCC GCCGCCGCCG CCGCCGCCGC CGCCGCCGCC GCTCCGCCCT

      3310        3320        3330        3340        3350        3360
GCTGTAGCCC CCTACGGCTA CACTCGGCCC CCTCAGGGGC TGGCGGGCCA GGAAAGCGAC
CGACATCGGG GGATGCCGAT GTGAGCCGGG GGAGTCCCCG ACCGCCCGGT CCTTTCGCTG

      3370        3380        3390        3400        3410        3420
TTCACCGCAC CTGATGTGTG GTACCCTGGC GGCATGGTGA GCAGAGTGCC CTATCCCAGT
AAGTGGCGTG GACTACACAC CATGGGACCG CCGTACCACT CGTCTCACGG GATAGGGTCA

      3430        3440        3450        3460        3470        3480
CCCACTTGTG TCAAAAGCGA AATGGGCCCC TGGATGGATA GCTACTCCCG GGAACCTTAC
GGGTGAACAC AGTTTTCGCT TTACCCGGGG ACCTACCTAT CGATGAGGGC CCTTGGAATG

      3490        3500        3510        3520        3530        3540
GGGGACATGC GTTTGGAGAC TGCCAGGGAC CATGTTTTGC CCATTGACTA TTACTTTCCA
CCCCTGTACG CAAACCTCTG ACGGTCCCTG GTACAAAACG GGTAACTGAT AATGAAAGGT

      3550        3560        3570        3580        3590        3600
CCCTAGAAGA CCTGCCTGAT CTGTGGAGAT GAAGCTTCTG GGTGTCACTA TGGAGCTCTC
GGGGTCTTCT GGACGGACTA GACACCTCTA CTTCGAAGAC CCACAGTGAT ACCTCGAGAG
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FIGURE 4 (page 5 of 6)

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      3610        3620        3630        3640        3650        3660
ACATGTGGAA GCTGCAAGGT CTTCTTCAAA AGAGCCGCTG AAGGGAAACA GAAGTACCTG
TGTACACCTT CGACGTTCCA GAAGAAGTTT TCTCGGCGAC TTCCCTTTGT CTTCATGGAC

      3670        3680        3690        3700        3710        3720
TGCGCCAGCA GAAATGATTG CACTATTGAT AAATTCCGAA GGAAAAATTG TCCATCTTGT
ACGCGGTCGT CTTTACTAAC GTGATAACTA TTTAAGGCTT CCTTTTTAAC AGGTAGAACA

      3730        3740        3750        3760        3770        3780
CGTCTTCGGA AATGTTATGA AGCAGGGATG ACTCTGGGAG CCCGGAAGCT GAAGAAACTT
GCAGAAGCCT TTACAATACT TCGTCCCTAC TGAGACCCTC GGGCCTTCGA CTTCTTTGAA

      3790        3800        3810        3820        3830        3840
GGTAATCTGA AACTACAGGA GGAAGGAGAG GCTTCCAGCA CCACCAGCCC CACTGAGGAG
CCATTAGACT TTGATGTCCT CCTTCCTCTC CGAAGGTCGT GGTGGTCGGG GTGACTCCTC

      3850        3860        3870        3880        3890        3900
ACAACCCAGA AGCTGACAGT GTCACACATT GAAGGCTATG AATGTCAGCC CATCTTTCTG
TGTTGGGTCT TCGACTGTCA CAGTGTGTAA CTTCCGATAC TTACAGTCGG GTAGAAAGAC

      3910        3920        3930        3940        3950        3960
AATGTCCTGG AAGCCATTGA GCCAGGTGTA GTGTGTGCTG GACACGACAA CAACCAGCCC
TTACAGGACC TTCGGTAACT CGGTCCACAT CACACACGAC CTGTGCTGTT GTTGGTCGGG

      3970        3980        3990        4000        4010        4020
GACTCCTTTG CAGCCTTGCT CTCTAGCCTC AATGAACTGG GAGAGAGACA GCTTGTACAC
CTGAGGAAAC GTCGGAACGA GAGATCGGAG TTACTTGACC CTCTCTCTGT CGAACATGTG

      4030        4040        4050        4060        4070        4080
GTGGTCAAGT GGGCCAAGGG CTTGCCTGGC TTCCGCAACT TACACGTGGA CGACCAGATG
CACCAGTTCA CCCGGTTCCC GAACGGACCG AAGGCGTTGA ATGTGCACCT GCTGGTCTAC

      4090        4100        4110        4120        4130        4140
GCTGTCATTC AGTACTCCTG GATGGGGCTC ATGGTGTTTG CCATGGGCTG GCGATCCTTC
CGACAGTAAG TCATGAGGAC CTACCCCGAG TACCACAAAC GGTACCCGAC CGCTAGGAAG

      4150        4160        4170        4180        4190        4200
ACCAATGTCA ACTCCAGGAT GCTCTACTTC GCCCCTGATC TGGTTTTCAA TGAGTACCGC
TGGTTACAGT TGAGGTCCTA CGAGATGAAG CGGGGACTAG ACCAAAAGTT ACTCATGGCG

      4210        4220        4230        4240        4250        4260
ATGCACAAGT CCCGGATGTA CAGCCAGTGT GTCCGAATGA GGCACCTCTC TCAAGAGTTT
TACGTGTTCA GGGCCTACAT GTCGGTCACA CAGGCTTACT CCGTGGAGAG AGTTCTCAAA

      4270        4280        4290        4300        4310        4320
GGATGGCTCC AAATCACCCC CCAGGAATTC CTGTGCATGA AAGCACTGCT ACTCTTCAGC
CCTACCGAGG TTTAGTGGGG GGTCCTTAAG GACACGTACT TTCGTGACGA TGAGAAGTCG

      4330        4340        4350        4360        4370        4380
ATTATTCCAG TGGATGGGCT GAAAAATCAA AAATTCTTTG ATGAACTTCG AATGAACTAC
TAATAAGGTC ACCTACCCGA CTTTTTAGTT TTTAAGAAAC TACTTGAAGC TTACTTGATG

      4390        4400        4410        4420        4430        4440
ATCAAGGAAC TCGATCGTAT CATTGCATGC AAAAGAAAAA ATCCCACATC CTGCTCAAGA
TAGTTCCTTG AGCTAGCATA GTAACGTACG TTTTCTTTTT TAGGGTGTAG GACGAGTTCT

      4450        4460        4470        4480        4490        4500
CGCTTCTACC AGCTCACCAA GCTCCTGGAC TCCGTGCAGC CTATTGCGAG AGAGCTGCAT
GCGAAGATGG TCGAGTGGTT CGAGGACCTG AGGCACGTCG GATAACGCTC TCTCGACGTA
```

FIGURE 4 (page 6 of 6)

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      4510       4520       4530       4540       4550       4560
CAGTTCACTT TTGACCTGCT AATCAAGTCA CACATGGTGA GCGTGGACTT TCCGGAAATG
GTCAAGTGAA AACTGGACGA TTAGTTCAGT GTGTACCACT CGCACCTGAA AGGCCTTTAC

      4570       4580       4590       4600       4610       4620
ATGGCAGAGA TCATCTCTGT GCAAGTGCCC AAGATCCTTT CTGGGAAAGT CAAGCCCATC
TACCGTCTCT AGTAGAGACA CGTTCACGGG TTCTAGGAAA GACCCTTTCA GTTCGGGTAG

      4630       4640       4650       4660       4670       4680
TATTTCCACA CCCAGTGAAG CATTGGAAAC CCTATTTCCC CACCCCAGCT CATGCCCCCT
ATAAAGGTGT GGGTCACTTC GTAACCTTTG GGATAAAGGG GTGGGGTCGA GTACGGGGGA

      4690       4700       4710       4720       4730       4740
TTCAGATGTC TTCTGCCTGT TATAACTCTG CACTACTCCT CTGCAGTGCC TTGGGGAATT
AAGTCTACAG AAGACGGACA ATATTGAGAC GTGATGAGGA GACGTCACGG AACCCCTTAA

      4750       4760       4770       4780       4790       4800
TCCTCTATTG ATGTACAGTC TGTCATGAAC ATGTTCCTGA ATTCTATTTG CTGGGCTTTT
AGGAGATAAC TACATGTCAG ACAGTACTTG TACAAGGACT TAAGATAAAC GACCCGAAAA

      4810       4820       4830       4840       4850       4860
TTTTTCTCTT TCTCTCCTTT CTTTTTCTTC TTCCCTCCCT ATCTAACCCT CCCATGGCAC
AAAAAGAGAA AGAGAGGAAA GAAAAAGAAG AAGGGAGGGA TAGATTGGGA GGGTACCGTG

      4870       4880       4890       4900       4910       4920
CTTCAGACTT TGCTTCCCAT TGTGGCTCCT ATCTGTGTTT TGAATGGTGT TGTATGCCTT
GAAGTCTGAA ACGAAGGGTA ACACCGAGGA TAGACACAAA ACTTACCACA ACATACGGAA

      4930       4940       4950       4960       4970       4980
TAAATCTGTG ATGATCCTCA TATGGCCCAG TGTCAAGTTG TGCTTGTTTA CAGCACTACT
ATTTAGACAC TACTAGGAGT ATACCGGGTC ACAGTTCAAC ACGAACAAAT GTCGTGATGA

      4990       5000       5010       5020       5030       5040
CTGTGCCAGC CACACAAACG TTTACTTATC TTATGCCACG GGAAGTTTAG AGAGCTAAGA
GACACGGTCG GTGTGTTTGC AAATGAATAG AATACGGTGC CCTTCAAATC TCTCGATTCT

      5050       5060       5070       5080
TTATCTGGGG AAATCAAAAC AAAAAACAAG CAAACAAAAA AAAAA
AATAGACCCC TTTAGTTTTG TTTTTTGTTC GTTTGTTTTT TTTTT
```

FIGURE 5 (page 1 of 5)

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        10          20          30          40          50          60
GAGCTCTGGA CAAAATTGAG CGCCTATGTG TACATGGCAA GTGTTTTTAG TGTTTGTGTG

        70          80          90         100         110         120
TTTACCTGCT TGTCTGGGTG ATTTTGCCTT TGAGAGTCTG GATGAGAAAT GCATGGTTAA

       130         140         150         160         170         180
AGGCAATTCC AGACAGGAAG AAAGGCAGAG AAGAGGGTAG AAATGACCTC TGATTCTTGG

       190         200         210         220         230         240
GGCTGAGGGT TCCTAGAGCA AATGGCACAA TGCCACGAGG CCCGATCTAT CCCTATGACG

       250         260         270         280         290         300
GAACTCTAAG GTTTCAGCAT CAGCTATCTG CTGGCTTGGT CACTGGCTTG CCTCCTCAGT

       310         320         330         340         350         360
TTGTAGGAGA CTCTCCCACT CTCCCATCTG CGCGCTCTTA TCAGTCCTGA AAAGAACCCN

       370         380         390         400         410         420
TGGCNAGCCA GGAGCNAGGT ATTCNTATCG TCCTTTTCNT CCTCCTNGCC TCACCTNGTT

       430         440         450         460         470         480
GNTTTTTAGA TTGGNCTTNG NAACCAAATT TGTATGCTGG CCTCCAGGAA ATCTGGAGCC

       490         500         510         520         530         540
TGGCGCCTAA ACCTTGGTTT AGGAAAGCAG GAGCTATTCA GGAAGCAGGG TCCTCCAGGG

       550         560         570         580         590         600
CTAGAGCTAG CCTCTCCTGC CCTCGCCCAC GTGCGCCAGC ACTTGTTTCT CCAAAGCNAC

       610         620         630         640         650         660
TAGGCAGGCG TTAGCGCGCG GTGAGGGGAG GGGAGAAAAG GAAAGGGGAG GGGAGGGAAA

       670         680         690         700         710         720
AGGAGGTGGG AAGGCAAGGA GGCCGGCCNG GTGGGGGCGG GACCCGACTC GCANNAACTG

       730         740         750         760         770         780
TTGCATTTGC TCTCCACCTC CCAGCGCCCC CTCCGAGATC CCGGGGAGCC AGCTTGCTGG

       790         800         810         820         830         840
GAGAGCGGGA ACGGTCCGGA GCAAGCCCAG AGGCAGAGGA GGCGACAGAG GGAAAAAGGG

       850         860         870         880         890         900
CCCNAGCTAG CCGCTCCAGT GCTGTACAGN AGCCGAAGGA CGCACCACGC CAGCCCCAGC

       910         920         930         940         950         960
CCGGCTCCAG CGACAGCNAA CGCCTCTTGC ANGCGTTCGA AGCCGCCGCC CGGAGCTGCC

       970         980         990        1000        1010        1020
CTTTCCTCTT CGGTGAAGTT TTTAAAAGCT GCTAAAGACT CGGAGGAAGC AAGGAAAGTG

      1030        1040        1050        1060        1070        1080
CCTGGTAGGA CTGACGGCTG CCTTTGTCCT CCTCCTCTCC ACCCCGCCTC CCCCCACCCT

      1090        1100        1110        1120        1130        1140
GCCTTCCCCC CCTCCCCCGT CTTCTCTCCC GCAGCTGCCT CAGTCGGCTA CTCTCAGCCA

      1150        1160        1170        1180        1190        1200
ACCCCCCTCA CCACCCTTCT CCCCACCCGC CCCCCCGCCC CCGTCGGCCC AGCGNTGNCA

      1210        1220        1230        1240        1250        1260
GNCCGAGTTT GCAGAGAGGT AACTCCCTTT AGCTGCGAGC GGGCGAGNCT AGCTGCACAT
```

FIGURE 5 (page 2 of 5)

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      1270        1280        1290        1300        1310        1320
TGCAAAGAAG GCTCTTAGGA GCAGGCGACT GGGGAGCGGC TTCAGCACTG CAGCCACGAC

      1330        1340        1350        1360        1370        1380
CNGCCTGGTT AGGCTGCACG CGGAGAGAAC CCTCTGTTTT CCCCCACTCT CTCTCCACCT

      1390        1400        1410        1420        1430        1440
CCTCCTGCCT TCCCCACCCC GAGTGCGGAG CCAGAGATCA AAAGATGAAA AGGCAGTCAG

      1450        1460        1470        1480        1490        1500
GTCTTCAGTA GCCAAAAAAC AAAACAAACA AAAACAAAAA AGCCGAAATA AAAGAAAAAG

      1510        1520        1530        1540        1550        1560
ATAATAACTC AGTTCTTATT TGCACCTACT TCAGTGGACA CTGAATTTGG AAGGTGGAGG

      1570        1580        1590        1600        1610        1620
ATTTTGTTTT TTTCTTTTAA GATCTGGGCA TCTTTTGAAT CTACCCTTCA AGTATTAAGA

      1630        1640        1650        1660        1670        1680
GACAGACTGT GAGCCTAGCA GGGCAGATCT TGTCCACCGT GTGTCTTCTT CTGCACGAGA

      1690        1700        1710        1720        1730        1740
CTTTGAGGCT GTCAGAGCGC TTTTTGCGTG GTTGCTCCCG CAAGTTTCCT TCTCTGGAGC

      1750        1760        1770        1780        1790        1800
TTCCCGCAGG TGGGCAGCTA GCTGCAGCGA CTACCGCATC ATCACAGCCT GTTGAACTCT

      1810        1820        1830        1840        1850        1860
TCTGAGCAAG AGAAGGGGAG GCGGGGTAAG GGAAGTAGGT GGAAGATTCA GCCAAGCTCA

                                  1890                                1900
GGG ATG GAA GTG CAG TTA GGG CTG GGA AGG GTC TAC CCT CGG CCG CCG TCC AAG ACC T-C
    Met Glu Val Gln Leu Gly Leu Gly Arg Val Tyr Pro Arg Pro Pro Ser Lys Thr Tyr

                                  1950                                1980
CGC GGA GCT TTC CAG AAT CTG TTC CAG AGC GTG CGC GAA GTG ATC CAG AAC CCG GGC CCC
Arg Gly Ala Phe Gln Asn Leu Phe Gln Ser Val Arg Glu Val Ile Gln Asn Pro Gly Pro

                                  2010                                2040
AGG CAC CCA GAG GCC GCG AGC GCA GCA CCT CCC GGC GCC AGT TTG CTG CTG CTG CAG CAG
Arg His Pro Glu Ala Ala Ser Ala Ala Pro Pro Gly Ala Ser Leu Leu Leu Leu Gln Gln

                                  2070                                2100
CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln

                                  2130                                2160
CAG CAG CAA GAG ACT AGC CCC AGG CAG CAG CAG CAG CAG CAG GGT GAG GAT GGT TCT CCC
Gln Gln Gln Glu Thr Ser Pro Arg Gln Gln Gln Gln Gln Gln Gly Glu Asp Gly Ser Pro

                                  2190                                2220
CAA GCC CAT CGT AGA GGC CCC ACA GGC TAC CTG GTC CTG GAT GAG GAA CAG CAA CCT TCA
Gln Ala His Arg Arg Gly Pro Thr Gly Tyr Leu Val Leu Asp Glu Glu Gln Gln Pro Ser

                                  2250                                2280
CAG CCG CAG TCG GCC CTG GAG TGC CAC CCC GAG AGA GGT TGC GTC CCA GAG CCT GGA GCC
Gln Pro Gln Ser Ala Leu Glu Cys His Pro Glu Arg Gly Cys Val Pro Glu Pro Gly Ala

                                  2310                                2340
GCC GTG GCC GCC AGC AAG GGG CTG CCG CAG CAG CTG CCA GCA CCT CCG GAC GAG GAT GAC
Ala Val Ala Ala Ser Lys Gly Leu Pro Gln Gln Leu Pro Ala Pro Pro Asp Glu Asp Asp
```

FIGURE 5 (page 3 of 5)

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                                   2370                                    2400
TCA GCT GCC CCA TCC ACG TTG TCC CTG CTG GGC CCC ACT TTC CCC GGC TTA AGC AGC TGC
Ser Ala Ala Pro Ser Thr Leu Ser Leu Leu Gly Pro Thr Phe Pro Gly Leu Ser Ser Cys

                                   2430                                    2460
TCC GCT GAC CTT AAA GAC ATC CTG AGC GAG GCC AGC ACC ATG CAA CTC CTT CAG CAA CAG
Ser Ala Asp Leu Lys Asp Ile Leu Ser Glu Ala Ser Thr Met Gln Leu Leu Gln Gln Gln

                                   2490                                    2520
CAG CAG GAA GCA GTA TCC GAA GGC AGC AGC AGC GGG AGA GCG AGG GAG GCC TCG GGG GCT
Gln Gln Glu Ala Val Ser Glu Gly Ser Ser Ser Gly Arg Ala Arg Glu Ala Ser Gly Ala

                                   2550                                    2580
CCC ACT TCC TCC AAG GAC AAT TAC TTA GGG GGC ACT TCG ACC ATT TCT GAC AAC GCC AAG
Pro Thr Ser Ser Lys Asp Asn Tyr Leu Gly Gly Thr Ser Thr Ile Ser Asp Asn Ala Lys

                                   2610                                    2640
GAG TTG TGT AAG GCA GTG TCG GTG TCC ATG GGC CTG GGT GTG GAG GCG TTG GAG CAT CTG
Glu Leu Cys Lys Ala Val Ser Val Ser Met Gly Leu Gly Val Glu Ala Leu Glu His Leu

                                   2670                                    2700
AGT CCA GGG GAA CAG CTT CGG GGG GAT TGC ATG TAC GCC CCA CTT TTG GGA GTT CCA CCC
Ser Pro Gly Glu Gln Leu Arg Gly Asp Cys Met Tyr Ala Pro Leu Leu Gly Val Pro Pro

                                   2730                                    2760
GCT GTG GCT CCC ACT CCT TGT GCC CCA TTG GCC GAA TGC AAA GGT TCT CTG CTA GAC GAC
Ala Val Ala Pro Thr Pro Cys Ala Pro Leu Ala Glu Cys Lys Gly Ser Leu Leu Asp Asp

                                   2790                                    2820
AGC GCA GGC AAG AGC ACT GAA GAT ACT GCT GAG TAT TCC CCT TTC AAG GGA GGT TAC ACC
Ser Ala Gly Lys Ser Thr Glu Asp Thr Ala Glu Tyr Ser Pro Phe Lys Gly Gly Tyr Thr

                                   2850                                    2880
AAA GGG CTA GAA GGC GAG AGC CTA GGC TGC TCT GGC AGC GCT GCA GCA GGG AGC TCC GGG
Lys Gly Leu Glu Gly Glu Ser Leu Gly Cys Ser Gly Ser Ala Ala Ala Gly Ser Ser Gly

                                   2910                                    2940
ACA CTT GAA CTG CCG TCT ACC CTG TCT CTC TAC AAG TCC GGA GCA CTG GAC GAG GCA GCT
Thr Leu Glu Leu Pro Ser Thr Leu Ser Leu Tyr Lys Ser Gly Ala Leu Asp Glu Ala Ala

                                   2970                                    3000
GCG TAC CAG AGT CGC GAC TAC TAC AAC TTT CCA CTG GCT CTG GCC GGA CCG CCG CCC CCT
Ala Tyr Gln Ser Arg Asp Tyr Tyr Asn Phe Pro Leu Ala Leu Ala Gly Pro Pro Pro Pro

                                   3030                                    3060
CCC CCG CCT CCC CAT CCC CAC GCT CGC ATC AAG CTG GAG AAC CCG CTG GAC TAC GGC AGC
Pro Pro Pro Pro His Pro His Ala Arg Ile Lys Leu Glu Asn Pro Leu Asp Tyr Gly Ser

                                   3090                                    3120
GCC TGG GCG GCT GCG GCG GCG CAG TGC CGC TAT GGG GAC CTG GCG AGC CTG CAT GGC GCG
Ala Trp Ala Ala Ala Ala Ala Gln Cys Arg Tyr Gly Asp Leu Ala Ser Leu His Gly Ala

                                   3150                                    3180
GGT GCA GCG GGA CCC GGT TCT GGG TCA CCC TCA GCC GCC GCT TCC TCA TCC TGG CAC ACT
Gly Ala Ala Gly Pro Gly Ser Gly Ser Pro Ser Ala Ala Ala Ser Ser Ser Trp His Thr

                                   3210                                    3240
CTC TTC ACA GCC GAA GAA GGC CAG TTG TAT GGA CCG TGT GGT GGT GGT GGG GGT GGT GGC
Leu Phe Thr Ala Glu Glu Gly Gln Leu Tyr Gly Pro Cys Gly Gly Gly Gly Gly Gly Gly
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FIGURE 5 (page 4 of 5)

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                                                3270                                                3300
GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GAG GCG GGA
Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Glu Ala Gly

                                                3330                                                3360
GCT GTA GCC CCC TAC GGC TAC ACT CGG CCC CCT CAG GGG CTG GCG GGC CAG GAA AGC GAC
Ala Val Ala Pro Tyr Gly Tyr Thr Arg Pro Pro Gln Gly Leu Ala Gly Gln Glu Ser Asp

                                                3390                                                3420
TTC ACC GCA CCT GAT GTG TGG TAC CCT GGC GGC ATG GTG AGC AGA GTG CCC TAT CCC AGT
Phe Thr Ala Pro Asp Val Trp Tyr Pro Gly Gly Met Val Ser Arg Val Pro Tyr Pro Ser

                                                3450                                                3480
CCC ACT TGT GTC AAA AGC GAA ATG GGC CCC TGG ATG GAT AGC TAC TCC CGG GAA CCT TAC
Pro Thr Cys Val Lys Ser Glu Met Gly Pro Trp Met Asp Ser Tyr Ser Arg Glu Pro Tyr

                                                3510                                                3540
GGG GAC ATG CGT TTG GAG ACT GCC AGG GAC CAT GTT TTG CCC ATT GAC TAT TAC TTT CCA
Gly Asp Met Arg Leu Glu Thr Ala Arg Asp His Val Leu Pro Ile Asp Tyr Tyr Phe Pro

                                                3570                                                3600
CCC CAG AAG ACC TGC CTG ATC TGT GGA GAT GAA GCT TCT GGG TGT CAC TAT GGA GCT CTC
Pro Gln Lys Thr Cys Leu Ile Cys Gly Asp Glu Ala Ser Gly Cys His Tyr Gly Ala Leu

                                                3630                                                3660
ACA TGT GGA AGC TGC AAG GTC TTC TTC AAA AGA GCC GCT GAA GGG AAA CAG AAG TAC CTG
Thr Cys Gly Ser Cys Lys Val Phe Phe Lys Arg Ala Ala Glu Gly Lys Gln Lys Tyr Leu

                                                3690                                                3720
TGC GCC AGC AGA AAT GAT TGC ACT ATT GAT AAA TTC CGA AGG AAA AAT TGT CCA TCT TGT
Cys Ala Ser Arg Asn Asp Cys Thr Ile Asp Lys Phe Arg Arg Lys Asn Cys Pro Ser Cys

                                                3750                                                3780
CGT CTT CGG AAA TGT TAT GAA GCA GGG ATG ACT CTG GGA GCC CGG AAG CTG AAG AAA CTT
Arg Leu Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Leu Lys Lys Leu

                                                3810                                                3840
GGT AAT CTG AAA CTA CAG GAG GAA GGA GAG GCT TCC AGC ACC ACC AGC CCC ACT GAG GAG
Gly Asn Leu Lys Leu Gln Glu Glu Gly Glu Ala Ser Ser Thr Thr Ser Pro Thr Glu Glu

                                                3870                                                3900
ACA ACC CAG AAG CTG ACA GTG TCA CAC ATT GAA GGC TAT GAA TGT CAG CCC ATC TTT CTG
Thr Thr Gln Lys Leu Thr Val Ser His Ile Glu Gly Tyr Glu Cys Gln Pro Ile Phe Leu

                                                3930                                                3960
AAT GTC CTG GAA GCC ATT GAG CCA GGT GTA GTG TGT GCT GGA CAC GAC AAC AAC CAG CCC
Asn Val Leu Glu Ala Ile Glu Pro Gly Val Val Cys Ala Gly His Asp Asn Asn Gln Pro

                                                3990                                                4020
GAC TCC TTT GCA GCC TTG CTC TCT AGC CTC AAT GAA CTG GGA GAG AGA CAG CTT GTA CAC
Asp Ser Phe Ala Ala Leu Leu Ser Ser Leu Asn Glu Leu Gly Glu Arg Gln Leu Val His

                                                4050                                                4080
GTG GTC AAG TGG GCC AAG GGC TTG CCT GGC TTC CGC AAC TTA CAC GTG GAC GAC CAG ATG
Val Val Lys Trp Ala Lys Gly Leu Pro Gly Phe Arg Asn Leu His Val Asp Asp Gln Met

                                                4110                                                4140
GCT GTC ATT CAG TAC TCC TGG ATG GGG CTC ATG GTG TTT GCC ATG GGC TGG CGA TCC TTC
Ala Val Ile Gln Tyr Ser Trp Met Gly Leu Met Val Phe Ala Met Gly Trp Arg Ser Phe
```

FIGURE 5 (page 5 of 5)

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                                                                4170                                                                4200
ACC AAT GTC AAC TCC AGG ATG CTC TAC TTC GCC CCT GAT CTG GTT TTC AAT GAG TAC CGC
Thr Asn Val Asn Ser Arg Met Leu Tyr Phe Ala Pro Asp Leu Val Phe Asn Glu Tyr Arg

                                                                4230                                                                4260
ATG CAC AAG TCC CGG ATG TAC AGC CAG TGT GTC CGA ATG AGG CAC CTC TCT CAA GAG TTT
Met His Lys Ser Arg Met Tyr Ser Gln Cys Val Arg Met Arg His Leu Ser Gln Glu Phe

                                                                4290                                                                4320
GGA TGG CTC CAA ATC ACC CCC CAG GAA TTC CTG TGC ATG AAA GCA CTG CTA CTC TTC AGC
Gly Trp Leu Gln Ile Thr Pro Gln Glu Phe Leu Cys Met Lys Ala Leu Leu Leu Phe Ser

                                                                4350                                                                4380
ATT ATT CCA GTG GAT GGG CTG AAA AAT CAA AAA TTC TTT GAT GAA CTT CGA ATG AAC TAC
Ile Ile Pro Val Asp Gly Leu Lys Asn Gln Lys Phe Phe Asp Glu Leu Arg Met Asn Tyr

                                                                4410                                                                4440
ATC AAG GAA CTC GAT CGT ATC ATT GCA TGC AAA AGA AAA AAT CCC ACA TCC TGC TCA AGA
Ile Lys Glu Leu Asp Arg Ile Ile Ala Cys Lys Arg Lys Asn Pro Thr Ser Cys Ser Arg

                                                                4470                                                                4500
CGC TTC TAC CAG CTC ACC AAG CTC CTG GAC TCC GTG CAG CCT ATT GCG AGA GAG CTG CAT
Arg Phe Tyr Gln Leu Thr Lys Leu Leu Asp Ser Val Gln Pro Ile Ala Arg Glu Leu His

                                                                4530                                                                4560
CAG TTC ACT TTT GAC CTG CTA ATC AAG TCA CAC ATG GTG AGC GTG GAC TTT CCG GAA ATG
Gln Phe Thr Phe Asp Leu Leu Ile Lys Ser His Met Val Ser Val Asp Phe Pro Glu Met

                                                                4590                                                                4620
ATG GCA GAG ATC ATC TCT GTG CAA GTG CCC AAG ATC CTT TCT GGG AAA GTC AAG CCC ATC
Met Ala Glu Ile Ile Ser Val Gln Val Pro Lys Ile Leu Ser Gly Lys Val Lys Pro Ile

                                                                4650                                                                4680
TAT TTC CAC ACC CAG TGA AGC ATT GGA AAC CCT ATT TCC CCA CCC CAG CTC ATG CCC CCT
Tyr Phe His Thr Gln End
```

```
      4690        4700        4710        4720        4730        4740
TTCAGATGTC TTCTGCCTGT TATAACTCTG CACTACTCCT CTGCAGTGCC TTGGGGAATT

      4750        4760        4770        4780        4790        4800
TCCTCTATTG ATGTACAGTC TGTCATGAAC ATGTTCCTGA ATTCTATTTG CTGGGCTTTT

      4810        4820        4830        4840        4850        4860
TTTTTCTCTT TCTCTCCTTT CTTTTTCTTC TTCCCTCCCT ATCTAACCCT CCCATGGCAC

      4870        4880        4890        4900        4910        4920
CTTCAGACTT TGCTTCCCAT TGTGGCTCCT ATCTGTGTTT TGAATGGTGT TGTATGCCTT

      4930        4940        4950        4960        4970        4980
TAAATCTGTG ATGATCCTCA TATGGCCCAG TGTCAAGTTG TGCTTGTTTA CAGCACTACT

      4990        5000        5010        5020        5030        5040
CTGTGCCAGC CACACAAACG TTTACTTATC TTATGCCACG GGAAGTTTAG AGAGCTAAGA

      5050        5060        5070        5080
TTATCTGGGG AAATCAAAAC AAAAAACAAG CAAACAAAAA AAAAA
```

FIGURE 6 (page 1 of 5)

```
                10         20         30         40         50         60
AATTCGGGAAGGATCGAECAAACCAGGAAAGTAAGGATGGAGCTCCTAGGAGAGTGTCCA

                70         80         90        100        110        120
TGCCTCGAAAGGAGCCCACCAAAGATGAACTGTTGCATTTGCTTTCCACCTCCCAGCGCC

               130        140        150        160        170        180
CCCTCGGAGATCCCTAGGAGCCAECCTGCTGGGAGAACCAGAGGGTCCGGAACAAACCTG

               190        200        210        220        250        240
GACCCTGAGAGGGCATCAGAGGGGAAAAGACTGAGTTAGCCACTCCAGTGCCATACAGAA

               250        260        270        280        290        300
GCTTAAGGGACATACCACGCCAGCCCCAGCCCAGCGACAGCCAACGCCTGTTGCAGAGCG

               310        320        330        340        350        360
GCGGCTTCGAAGCCGCCGCCCAGAAGCTGCCCTTTCCTCTTCGGTGAAGTTTCTAAAAGC

               370        380        390        400        410        420
TCCGGCAGACTCGGAGGAAGCGAAGAAAGTGTCCGGTAGGACTACGACTCCCTTTGTCCT

               430        440        450        460        470        480
CCTCCCTCCTACCCCTACCCCTCCTGGCTCCCCTCTCCCTGAGCGGACTAGGCAGGCTTC

               490        500        510        520        530        540
CTGGCCAGCCCTCTCCCCTACACCACCAGCTCTGCCAGCCAGTTTGCACAGAGGTAACTC

               550        560        570        580        590        600
CCTTTGGCTGAAAGCAGACGAGCTTGTTGCCCATTGGAAGGGAGGCTTTTGGGAGCCCAG

               610        620        630        640        650        660
AGACTGAGGAGCAACAGCACGCTGGAGAGTCCCTGATTCCAGGTTCTCCCCCCTGCACCT

               670        680        690        700        710        720
CCTACTGCCCCCCCCTCACCCTGTGTGTGCAGCTAGAATTGAAAAGATGAAAAGACAGTT

               730        740        750        760        770        780
GGGGCTTCAGTAGTCGAAAGCAAAACAAAGCAAAAGGAAAACCAAAAACGAAAATAGCCCA

               790        800        810        820        830        840
GTTCTTATTTGCACCTGCTTCAGTGGACATTGACTTTGGAAGCCAGAGAATTTTCCTTCC

               850        860        870        880        890        900
CCCCAGTCGAAGCTTTGAGCATCTTTTAATCTGTTCTTCAAGTATTTAGGGACAACTGTG
```

FIGURE 6 (page 2 of 5)

FIGURE 6 (page 3 of 5)

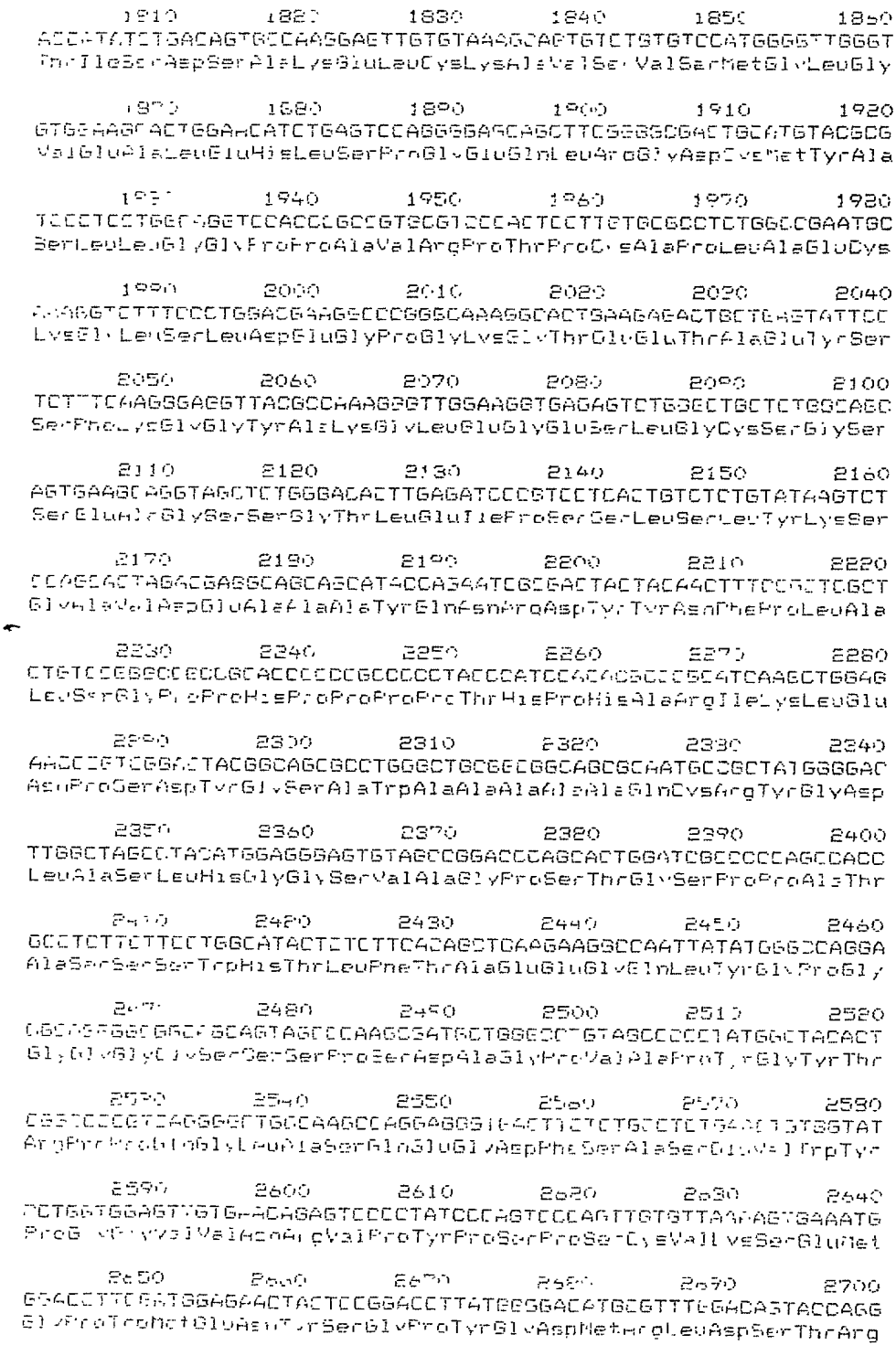

FIGURE 6 (page 4 of 5)

FIGURE 6 (page 5 of 5)

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      3610        3620        3630        3640        3650        3660
TGCAAAGAAAAAAATCCCACATCCTGCTCAAGGCGCTTCTACCAGCTCACCAAGCTCCTG
CysLysArgLysAsnProThrSerCysSerArgArgPheTyrGlnLeuThrLysLeuLeu

      3670        3680        3690        3700        3710        3720
GATTCTGTGCAGCCTATTGCAAGAGAGCTGCATCAATTCACTTTTGACCTGCTAATCAAG
AspSerValGlnProIleAlaArgGluLeuHisGlnPheThrPheAspLeuLeuIleLys

      3730        3740        3750        3760        3770        3780
TCCCATATGGTGAGCGTGGACTTTCCTGAAATGATGCCAGAGATCATCTCTGTGCAAGTG
SerHisMetValSerValAspPheProGluMetMetAlaGluIleIleSerValGlnVal

      3790        3800        3810        3820        3830        3840
CCCAAGATCCTTTCTGGGAAAGTCAGCCCATGTATTTCTACACACAGTGAAGATTTGGAA
ProLysIleLeuSerGlyLysValSerProCysIleSerThrHisSerGluAspLeuGlu

      3850        3860        3870        3880        3890        3900
CCTAATACCCAAACCCACCTGTTCCCTTTTCAGATGTCTTCTGCCTGTTATATAACTCTG
ProAsnThrGlnThrHisLeuPheProPheGlnMetSerSerAlaCysTyrIleThrLeu

      3910        3920        3930        3940        3950        3960
CACTACTTCTCTGGCATGGGCTTGGGGGAAATTCCTCTACTGATGTACAGTCTGTCATG
HisTyrPheSerGlyMetGlyLeuGlyGlyAsnSerSerThrAspValGlnSerValMet

      3970        3980        3990        4000        4010        4020
AACATGTTCCCCAAGTTCTATTTCCTGGGCTTTTCCTTCTTTCTTTTTCTTCTTCTCTGC
AsnMetPheProLysPheTyrPheLeuGlyPheSerPhePheLeuPheLeuLeuLeuCys

      4030        4040        4050        4060        4070        4080
CTCTTTTACCCTCCCATGGCACATTTTGAATCCGCTGCGTGTTGTGGCTCCTGCCTGTGT
LeuPheTyrProProMetAlaHisPheGluSerAlaAlaCysCysGlySerCysLeuCys

      4090        4100        4110        4120        4130        4140
TTTGAGTTTTGTTGTATTTCTTCAAGTCTGTGATGATCTTCTTGTGGCCCAGTGTCAACT
PheGluPheCysCysIleSerSerSerLeuEnd

      4150        4160        4170        4180        4190        4200
GTGCTTGTTTATAGCACTGTGCTGTGTGCCAACCAAGCAAATGTTTTACTCACCTTATGCC

      4210        4220        4230        4240        4250        4260
ATGGCAAGTTTAGAGAGCTATAAGTATCTTGGGAAGAAACAAACAGAGAGAGTAAAAAAA

      4270        4280
CCAAAAAAAAAAAAAAAAAACCGAATTC
```

FIGURE 7

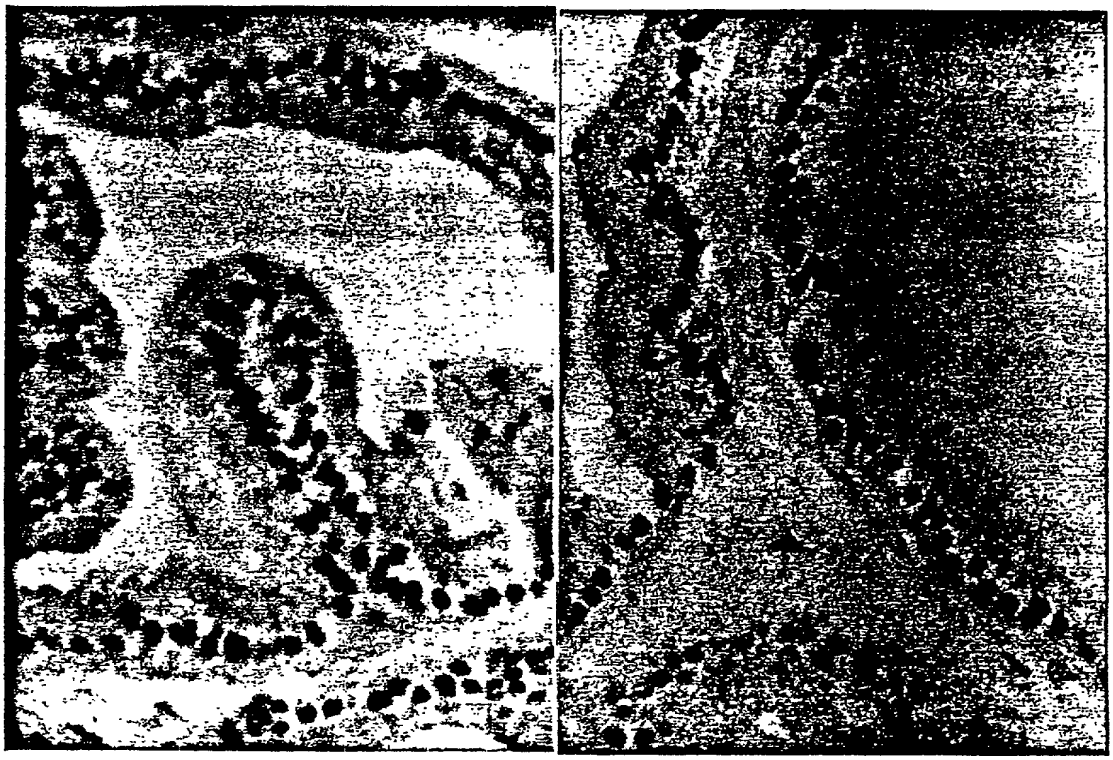

Figure 7. Frozen section of rat ventral prostate stained with antibodies (AR-52-3-p) to the AR peptide NH_2-Asp-His-Val-Leu-Pro-Ile-Asp-Tyr-Tyr-Phe-Pro-Pro-Gln-Lys-Thr in a dilution of 1 to 3000 using the avidin-biotin peroxidase technique. Androgen receptor is indicated by brown staining of nuclei in epithelial cells. Immuno-staining was performed as previously described (60).

FIGURE 8

Restriction Fragment Length Polymorphism in the Human Androgen Receptor Gene

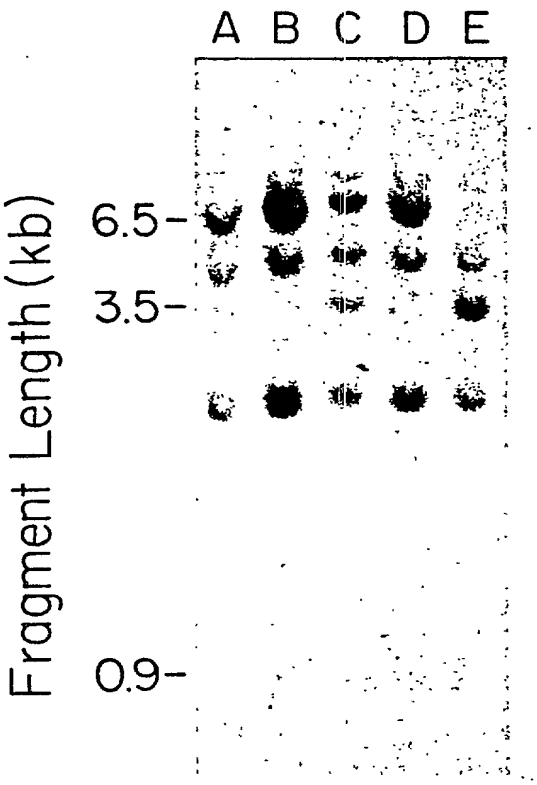

FIGURE 9

Southern Blot Analysis of Complete Androgen Insensitivity Syndrome Patients